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(57) Abstract

The present invention provides isolated peptides and combinations of peptides derived form myelin autoantigens such as MBP, MOG, PLP, and MAG suitable for treating multiple sclerosis, including prophylactic and therapeutic compositions and methods for preventing or treating multiple sclerosis. Preferred compositions of the invention comprise at least one isolated, purified peptide, free from all other polypeptides or contaminants, the peptide comprising an amino acid sequence, the myelin autoantigen which has T cell activity. A therapeutic composition of the invention is capable of down regulating the autoantigen specific immune response to the myelin autoantigen in a population of humans suffering from, or susceptible to multiple sclerosis, such that disease symptoms are reduced, eliminated, or reversed and/or the onset or progression of disease symptoms is prevented or slowed. Additionally, compositions and methods of the instant invention when administered in an advanced stage of disease, reverse ongoing paralysis or other signs of disease when administered during the acute phase of disease or prevents relapse when administered during remission.

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Compositions and Treatment for Multiple Sclerosis

Related Applications

This application is a continuation-in-part of USSN 08/328,224 filed October 25, 1994. This application is also a continuation in part of USSN 08/300,811 filed September 1, 1994 which is a continuation-in-part of USSN 08/116,824 filed September 3, 1993. This application is also a continuation-in-part of USSN 08/241,246 filed May 10, 1994. The above applications are hereby incorporated herein by reference in their entirety.

Background of the Invention

Autoimmune diseases are a significant human health problem and are relatively poorly understood. As there is no microbial or viral culprit apparently directly responsible, prevention, treatment and diagnosis of such diseases must be based on the etiology of the disease. This invariably involves a complex series of reactions of endogenous metabolic intermediates, structural components, cells and so forth. Implicit however in the nature of an autoimmune condition is the notion that at least one autoantigen must be involved in creating the sequence of events that results in the symptoms. Autoimmune demyelinating diseases such as multiple sclerosis are no exception.

MS usually presents in the form of recurrent attacks reflecting lesions within the central nervous system (CNS). Attacks recur, remit and recur, seemingly randomly over many years. The frequency of flare-ups is greatest during the first 3 to 4 years of disease, but a first attack, which may have been so mild as to have escaped medical attention and can barely be recalled, may not be followed by another attack for 10-20 years. The extent of recovery after an episode varies markedly between patients. Remission may be complete, particularly after early attacks; often, however remission is incomplete and as one attack follows another, a stepwise downward progression ensues with increasing permanent deficit. The clinical picture of MS is determined by the location of foci of demyelination within the CNS. Classic features include impaired vision, nystagmus, dysarthria, decreased perception of vibration and position sense, ataxia and intention tremor, weakness or paralysis or one or more limbs, spasticity and bladder problems. Criteria for diagnosis of clinically definite MS must include a reliable history of at least two episodes of neurological deficit and

objective clinical sign of lesion at more than one site within the CNS. No effective treatment for MS is known. Present therapeutic efforts are directed towards amelioration of the acute episode and prevention of relapses or progression of the disease. (*Harrison's Principles of Internal Medicine*, 12th Edition, Vol. 2, pp. 2038-2043, McGraw Hill, 1991)

A commonly used animal model for human multiple sclerosis is experimental allergic encephalomyelitis (EAE), a demyelinating disease of the central nervous system which can be induced in susceptible strains of mice by immunization with myelin basic protein (MBP), proteolipid protein (PLP), myelin oliogodendrocyte protein (MOG), or synthetic peptides based on the sequences of these myelin associated proteins. MBP is one of the presumed autoantigens in multiple sclerosis (MS) and has been epitope-mapped in both human (Ota et al., *Nature*, **346**:183-187 (1990)) and rodent (Zamvil et al., *Nature*, **324**:258-260 (1986)) systems. Peptides which are believed to comprise at least one T cell epitope of MBP (myelin basic protein), have been identified in WO 93/21222, EP 0 304 279, WO 91/15225, Ota et al, *Letters to Nature*, **346**:183-187 (1990), Wucherpfennig et al., *J. Exp. Med.*, **170**:279-290 (1994). Other MBP T cell epitope-containing peptides have been identified in applications which are incorporated in their entirety herein by reference U.S.S.N. 08/328,224 filed on October 25, 1994 and U.S.S.N. 08/241,246 filed on May 10, 1994. MOG peptides having T cell activity have been identified in USSN 08/300,811 incorporated by reference herein.

Proteolipid protein (PLP) and myelin associated glycoprotein (MAG) have also been implicated as possible autoantigens in multiple sclerosis. Studies describing the pathogenesis of the autoimmune response to PLP in multiple sclerosis have been described in Trotter et al., *J. Neuroimmunol.*, **33**:55-62 (1991); T cell epitopes of PLP have been described in Pelfrey et al., *J.Neuroimmunol.*, **46**:33-42 (1993). Studies describing MAG as a potential autoantigen in multiple sclerosis are described in Johnson et al., *J. Neuroimmunol.*, **13**:99-108 (1986).

Experimental autoimmune encephalomylitis (EAE) is a CD4+ T cell-mediated autoimmune disease which resembles multiple sclerosis in some of its clinical and histological features, and serves as an experimental model for this and other autoimmune diseases. EAE is an inflammatory disease of the central nervous system, resulting in paralysis and other neurologic abnormalities. It is typically induced with purified myelin proteins and peptides. Nevertheless, the EAE model has been used extensively to examine mechanisms of autoimmunity, and to investigate potential therapeutics for autoimmune disease.

As early as 1965, the observation was made that EAE could be treated by administration of MBP in a non-encephalitogenic form, presumably by the induction of immunological non-responsiveness, or tolerance (Alvord, E.C., et al., *Ann. NY Acad Sci.*, 1965, **122**:333, Levine, S.E., et al., *Science*, 1968, **161**:1155, Bernard, C.C. A., *1977 Clin. Exp. Immunol.*, 1977, **29**:100). This early observation has been extended over the past several years, with a number of investigators showing that neonatal or adult administration of MBP and MBP peptides prevents EAE (Bernard, C.C. A., *1977 Clin. Exp. Immunol.*, 1977, **29**:100, Clayton, J.P., et al., *J. Exp. Med.*, 1989, **169**:1681, Smilek, D.E., et al., *Proc. Nat'l. Acad. Sci.*, 1991, **88**:9633, Guar, A., et al., *Science*, 1992, **258**:1491, Metzler, B. et al., *Int. Immunol*, 1993, **5**:1159, Miller, A., et al., *J. Neuroimmunol.*, 1993, **46**:73, Critchfield, J.M., et al., *Science*, 1994, **263**:1139, Miller, A., et al., *Proc. Nat'l. Acad. Sci.*, USA, 1992, **89**:421). These studies suggest various routes of administration which include subcutaneous, intraperitoneal, intranasal, intravenous, and oral. Induction of immunological non-responsiveness in adults animals with intravenous peptides has been demonstrated in a variety of antigen systems. For many of the reasons described herein, there are limits to the clinical applicability of oral, enteral or aerosol administration of autoantigens such as an inability to characterize the active component of a therapeutic composition once introduced in the stomach due to subsequent enzymatic degradation in the stomach. Thus, predictable and reproducible therapeutic effects may be difficult to achieve using these methods, not to mention the potential for adverse side effects as a result of the body's further processing of the therapeutic which may not be predictable.

The mechanism of disease prevention or tolerance induction in most of these examples has been attributed to clonal anergy (Gaur et al., *supra*), peripheral deletion (Critchfield, et al. *supra*), or other forms of antigen-specific tolerance. However, TGF-β-mediated bystander suppression appears to be an additional mechanism by which orally administered MBP and MBP peptides may inhibit EAE (Miller et al., *supra* at *Proc. Nat'l Acad. Sci.*). MBP peptides as well as substituted MBP peptide analogs have been examined as alternative therapeutics for EAE in PLJ, B10.PL, and (PLJ x SJL) F1 mice. MBP Ac1-11 is the immunodominant encephalitogenic peptide for each of these strains, and is recognized bound to $A\alpha^{u}A\beta^{u}$ (Wraith, D.C. et al., *supra*). MBP Ac1-11 has been studied extensively by substitution analysis, and its requirements for T cell recognition and major histocompatibility complex (MHC) binding have been well established. Side chains of residues 3 and 6 contribute mainly to T cell recognition, while those of residues 4 and 5 contribute mainly to MHC binding. Binding of MBP Ac1-11 to $A\alpha^{u}A\beta^{u}$ can be dramatically improved by a

variety of amino acid substitutions at residue 4, including alanine (Ac1-11[4A]) and tyrosine (Ac1-11[4Y]) (Wraith, D.C. et al., *supra*, Fairchild, P.J. et al., *Int. Immunol.*, 1993, 5:1151). The residue 4 substitutions, especially with tyrosine, appear to improve the stability of the peptide-MHC complexes formed with these peptides. Ac1-11[4A] and Ac1-11[4Y] retain the T cell receptor (TCR) contact residues necessary for antigenicity, and are more potent than MBP Ac1-11 in stimulating MBP-specific T cells *in vitro*. Ac1-11[4Y] is also encephalitogenic *in vivo* (Ac1-11[4A] is poorly encephalitogenic, for unknown reasons). Both Ac1-11[4A] and Ac1-11[4Y] have been shown to prevent EAE, and are thought to operate by antigen-specific mechanisms (Smilek, et al, *supra*, Wraith, D.C. et al., *supra*).

In previous studies, MBP peptides or peptide analogs administered in incomplete adjuvant just prior to disease onset prevented subsequent development of EAE (Smilek, et al, *supra*, Gaur et al., *supra*). In a separate study using lymphocytes from an MBP-specific TCR transgenic mouse, adoptively transferred EAE was prevented by early and aggressive administration of intravenous MBP prior to the onset of clinical signs (Critchfield, et al. *supra*). These studies indicated that it may be possible to prevent EAE by injecting MBP peptides after encephalitogenic T cells have been activated, but did not address the issue of whether ongoing paralysis (and presumably active central nervous system inflammation) could be reversed, or relapses following remission could be prevented, using this approach. Moreover, in some of the previous experiments by others, frequent administration of extremely large doses of MBP or MBP peptide were required for effective treatment. The present inventors were the first to describe reversal of paralysis as well as prevention of relapses following remission in their previous case, USSN 08/328,224 filed October 25, 1994, which has been continued herein. Since the filing of the present inventor's previous case, others working in the field have also been surprised to find that injection of certain MBP derived peptide analogs could reverse ongoing paralysis in EAE (Karin et al., *J. Exp. Med.*, **180**:2227-2237 (Dec. 1994)).

The present invention overcomes the drawbacks described above and provides novel peptides, compositions and methods for treating multiple sclerosis, using preparations comprising at least one peptide having a sequence of amino acid residues which comprises T cell activity of MBP. Further, the present invention addresses the yet unsolved problem that, in order to be generally applicable, treatments for multiple sclerosis using peptides or peptide analogs must be effective even when administered late in the course or at an advanced stage of disease, either during remissions or relapses while the undesirable immune response is ongoing.

Accordingly, it is an object of the present invention to provide peptides and combinations of peptides suitable as a therapeutic for multiple sclerosis including prevention of the onset of the disease. It is yet another object of this invention to identify prophylactically and therapeutically effective dosage regimens and modes of administration of identified proteins, peptides and peptide analogs for effective treatment of MS. It is still another object of this invention to identify treatment which successfully treats late stage MS, prevent relapses, arrest disease and/or reverse the progression of MS.

Summary of the Invention:

The present invention provides isolated peptides and combinations of peptides derived from myelin autoantigens such as MBP, MOG, PLP, and MAG suitable for treating multiple sclerosis, including prophylactic and therapeutic compositions and methods for preventing or treating multiple sclerosis. Preferred compositions of the invention comprise at least one isolated, purified peptide, substantially free from all other polypeptides or contaminants, the peptide comprising an amino acid sequence of the myelin autoantigen which has T cell activity. A therapeutic composition of the invention is capable of down regulating the autoantigen specific immune response to the myelin autoantigen in a population of humans suffering from, or susceptible to multiple sclerosis, such that disease symptoms are reduced, eliminated, or reversed and/or the onset or progression of disease symptoms is prevented or slowed. Additionally, compositions and methods of the instant invention when administered in an advanced stage of disease, reverse ongoing paralysis or other signs of disease when administered during the acute phase of disease or prevents relapse when administered during remission.

Brief Description of the Drawings

Fig. 1 shows the full length amino acid sequence of human MBP, also indicating the numbering of amino acid residues as referred to herein.

Fig. 2 shows the amino acid sequence of preferred peptides derived from MBP.

Fig. 3 shows the amino acid sequence of overlapping peptides as well as longer peptides used in Example 1.

Fig. 4a is a graphic representation of the percent of total MBP response for each peptide shown in Fig. 3. MBP reactivity was calculated based on the percent of MBP positive microtiter cultures in each group of individual patients which also

scored positive for one of the MBP peptides. Total MBP positive microtiter cultures ranged from 89-184 in each group of 19-43 patients tested for each peptide.

Fig. 4b is a graphic representation of the percent of MBP responders which recognize each peptide shown in Fig. 3. Patients were considered to MBP responders if at least one of their microtiter cultures scored positive for MBP reactivity. There were 12-31 MBP responder patients in each group of 19-43 patients tested for each peptide. Peptide reactivity was calculated based on the percent of MBP responder patients in each group with at least one microtiter culture which scored positive for one of the MBP peptides.

Fig. 5a is a graph of mean clinical scores over a period of 0-40 days after disease induction of mice treated with I.V. injections of MBP Ac1-11 alone compared to a control.

Fig. 5b is a graph of mean clinical scores over a period of 0-40 days after disease induction of mice treated with I.V. injections of MBP Ac1-11 combined with MBP 31-47 compared to a control.

Fig. 5c is a graph of mean clinical scores over a period of 0-40 days after disease induction of mice treated with I.V. injections of OVA 323-339 compared to a control.

Fig. 6a is a graph of the mean clinical scores over a period of 0 to at least 30 days after disease induction of subjects treated with either 250 nmol injection of Ac1-11[4Y] or Ac 1-11 as compared to a control.

Fig. 6b is a graph of the mean clinical scores over a period of 0 to at least 30 days after disease induction of mice treated with 2.5 nmol Ac1-11[4Y] as compared to a control.

Fig. 6c is a graph of the mean clinical scores over a period of 0 to at least 30 days after disease induction of mice treated with 2.5 nmol Ac 1-11 as compared to a control.

Fig. 7 is a bar graph showing the mean histological scores of peptide treated mice versus control treated mice where a lower score means a reduced number and severity of inflammatory CNS infiltrates.

Fig. 8 is a bar graph representing relative T cell activation at periods of 0-10 hours after mice were injected with 250 nmol. Ac1-11[4Y].

Fig. 9 is a graph showing a comparison of treatment of mice with 250 nmol Ac1-11[4Y] versus a control, PBS and OVA 323-337.

Fig. 10 is a graph showing a comparison of treatments initiated during remission with 25 nmol Ac1-11[4Y] versus a control.

Fig. 11a is a graph representing *in vitro* lymph node proliferation (as indicated by 3H-thymidine incorporation) of mice receiving I.V. pretreatment with 250 nmoles MBP Ac1-11 compared to controls.

Fig. 11b is a bar graph showing relative lymph node IL2 production in response to MBP Ac1-11 in mice pretreated with Ac1-11[4Y] as compared to a control.

Fig. 12a is a graphic depiction of an experiment showing the effects of IFN-β on EAE in two groups of 10 (SJL x TLP) F_1 adult female mice in which EAE was induced with guinea pig MBP in complete adjuvant plus pertussis toxin on day 0 and were administered either incomplete Freund's Adjuvant only (control) or were treated with 2000 units of IFN-β interperitoneally (i.p.) on days 9, 12, 16, and 20 (indicated by arrows on the x axis), the Y axis represents the mean clinical score (MCS) for each group, 0=no clinical signs of EAE, 1=limp, unresponsive tail, 2=partial hind limb paralysis, 3=complete hind limb paralysis, 4=partial to complete forelimb paralysis and 5=moribund.

Fig. 12b is a graphic depiction of an experiment showing the effects of MBP peptide Ac 1-11 in two groups of 10 (SJL x TLP) F_1 adult female mice induced with EAE using guinea pig MBP in complete adjuvant plus pertussis toxin on day 0 and were administered either PBS (control) or were treated with 250 nmol Ac 1-11 intravenously on days 10, 13, 17, and 21 (indicated by arrows on the x axis), the Y axis represents the average mean clinical score for each group as described for Fig. 1a.

Fig. 12c is a graphic depiction of an experiment showing the effects of MBP peptide Ac 1-11 administered in combination with IFN-β on EAE in two groups of 10 (SJL x TLP) F_1 adult female mice who were induced EAE in complete adjuvant plus pertussis toxin on day 0 and were administered either PBS (control) or were treated with 250 nmol Ac 1-11 intravenously on days 10, 13, 17, and 21 (indicated by open arrows on the x axis), and were treated with 2000 units of IFN-β interperitoneally (i.p.) on days 9, 12, 16, and 20 (indicated by closed arrows on the x axis), the Y-axis indicates the mean clinical score as discussed for Fig. 1a.

Fig. 13 is a graphic depiction of an experiment showing the effects of various dose of IFN-β (10,000 units and 2,000 units respectively) on induced EAE in two groups of 10 (SJL x TLP) F_1 adult female mice induced with EAE using guinea pig MBP plus pertussis toxin on day 0 and were administered either PBS (control) or were treated with 10,000 units and 2000 units respectively of IFN-β interperitoneally (i.p.) on days 9, 13, 16, (indicated by closed arrows on the x axis), the Y-axis indicates the mean clinical score as discussed for Fig. 1a.

Fig. 14 shows various peptides derived from MBP which may be suitable in compositions and methods of the invention.

Fig. 15 is a graphic representation of the positivity index (y axis) (the average S.I./MBP responder patient) multiplied the percentage of individuals responding to each peptide (x-axis) which is derived from the same experimental data shown in Figs. 4a and 4b and described in Example 1.

Detailed Description of the Invention

The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. The issued U.S. patents, PCT publications, and other publications cited herein are hereby incorporated by reference.

The present invention provides isolated peptides and combinations thereof derived from myelin autoantigens useful for treating multiple sclerosis as well as therapeutic compositions and methods for treating multiple sclerosis. As used herein the term "treating multiple sclerosis" includes: prophylactic treatment of those mammals susceptible to MS; treatment at the initial onset of MS; and treatment of all "advanced stage" MS including relapsing-remitting MS, chronic progressive MS, primary progressive MS and benign MS. Therapeutic compositions of the invention comprise at least one purified peptide, substantially free from all other proteins or contaminants, and comprising a defined sequence of amino acid residues of a myelin antigen having T cell stimulating activity, which peptide may also be an isolated peptide. As used herein, the term "isolated" refers to a peptide which is free of all other polypeptides, contaminants, starting reagents or other materials, and which is unconjugated to any other molecule.

In accordance with this invention, a "peptide" refers to a defined sequence of amino acid residues which is less than the amino acids of the native protein antigen. A peptide of the invention preferably comprises at least approximately seven amino acid residues in length, and preferably at least about 12-40 amino acid residues in length, and more preferably at least 13-30 amino acid residues in length and which, when derived from a protein antigen, contains less than the amino acids of the entire protein antigen and preferably no more than about 75% of the amino acid residues of the entire protein antigen. Peptides used in accordance with the invention have T cell activity. A peptide having "T cell activity" may possess any one or more of the following characteristics: a) the ability to elicit a T cell response, such as stimulation (i.e. proliferation or lymphokine secretion); b) the ability to cause T cell non responsiveness or reduced T cell responsiveness of appropriate T cell subpopulations

such that they do not participate in stimulating an immune response to the offending autoantigen (e.g. via anergy, tolerance, or apoptosis); c) the ability to modify the lymphokine secretion profile as compared with exposure to the naturally occurring autoantigen; d) the ability to cause induction of T suppressor cells; and e) is capable of causing down regulation of autoimmune disease symptoms by any mechanism; or f) is derived from a bystander antigen and possesses the ability to elicit suppressor T cells at the site of myelin autoimmune attack which in turn results in down regulating the immune responses in the locality of the myelin autoimmune attack.

Peptides comprising at least one T cell epitope have T cell activity and are capable of eliciting a T cell response such as T cell stimulation (i.e. T cell proliferation or lymphokine secretion) and/or are capable of down regulating the autoantigen specific T cell response which may result in autoantigen specific T cell non-responsiveness or a reduced level of autoantigen specific T cell responsiveness. A T cell epitope is the basic element or smallest unit of recognition by a T cell receptor, where the epitope comprises amino acids essential to receptor recognition. T cell epitopes are believed to be involved in the initiation and perpetuation of the immune response to an antigen or autoantigen. These T cell epitopes are thought to trigger early immune response events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. These events lead to T cell proliferation, lymphokine secretion, local inflammatory reactions, recruitment of additional immune cells to the site, and activation of the B cell cascade leading to production of antibodies. In the case of an autoimmune disease, the antibodies produced are autoantibodies against an autoantigen such as MBP resulting in the clinical symptoms of an autoimmune disease.

Peptides having defined amino acid compositions and which comprise T cell epitopes may be identified for any myelin autoantigen, including MBP. One method includes dividing the protein antigen into non-overlapping, or overlapping peptides of desired lengths and synthesizing, purifying and testing those peptides to determine whether the peptides comprise at least one T cell epitope of MBP using any number of assays (i.e. T cell proliferation assays, lymphokine secretion assays, and T cell non-responsiveness studies). In another method an algorithm is used for predicting those peptides which are likely to comprise T cell epitopes and then synthesizing, purifying and testing the peptides predicted by the algorithm in T cell assays or *in vivo* studies to determine if such predicted peptides cause T cell proliferation or lymphokine secretion, or T cell non-responsiveness and are therefore likely to

contain T cell epitopes. As discussed in many of the documents cited above, human T cell activity can be tested by culturing T cells obtained from an individual sensitive to an autoantigen such as MBP with a peptide derived from the antigen and determining whether proliferation of T cells occurs in response to the peptide as measured, e.g., by cellular uptake of tritiated thymidine. Stimulation indices for responses by T cells to peptides can be calculated as the maximum counts per minute (CPM) in response to a peptide divided by the control CPM. A T cell stimulation index (S.I.) equal to or greater than two times, and preferably three times, the background level is considered "positive". Positive results are used in the analysis of a peptides potential therapeutic effectiveness as discussed later herein and in Example 1. Preferred peptides useful in accordance with this invention comprise at least one T cell epitope and preferably at least two or more T cell epitopes.

One algorithm for predicting peptides having T cell stimulating activity is reported in Rothbard, *1st Forum in Virology, Annals of the Pasteur Institute*, pp 518-526 (December, 1986), Rothbard and Taylor, *Embo*, 7:93-100 (1988), and EP 0 304 279. These documents report defining a general pattern (algorithm) for binding of a peptide to class II MHC, its statistical significance and the correlation of the pattern with known T cell epitopes as well as its successful use in predicting previously unidentified T cell epitopes of various protein antigens and autoantigens. The general pattern for a peptide known to bind Class II MHC well as reported in the above-mentioned documents appears to contain a linear pattern composed of a charged amino acid residue or glycine followed by two hydrophobic residues. After determining if a peptide conforms to the general pattern, the peptide can then be tested for T cell reactivity. Other algorithms that have been used to predict T cell epitopes of previously undefined proteins include an algorithm reported by Margalit et al., *J. Immunol.*, **138**:2213-2229 (1987), which is based on an amphipathic helix model.

Additionally peptides comprising "cryptic T cell epitopes" may be determined and are also useful in the methods and compositions of the invention. Cryptic T cell epitopes are those determinants in a protein antigen which, due to processing and presentation of the native protein antigen to the appropriate MHC molecule, are not normally revealed to the immune system. However, a peptide comprising a cryptic T cell epitope is capable of causing T cells to become non-responsive, and when a subject is primed with the peptide, T cells obtained from the subject will proliferate *in vitro* in response to the peptide or the protein antigen from which the peptide is derived. Peptides which comprise at least one cryptic T cell epitope derived from a protein antigen are referred to herein as "cryptic peptides". To confirm the presence

of cryptic T cell epitopes a T cell proliferation assay may be used as is known in the art in which antigen primed T cells are cultured *in vitro* in the presence of each peptide separately to establish peptide-reactive T cell lines. A peptide is considered to comprise at least one cryptic T cell epitope if a T cell line can be established with a given peptide and T cells are capable of proliferation upon challenge with the peptide and the protein antigen from which the peptide is derived.

In addition, it is not necessary that a peptide used in accordance with the method of this invention be derived from the amino acid sequence of the myelin autoantigen such as MBP. Any peptide comprising a defined sequence of amino acid residues and which is capable of down-regulating an antigen specific immune response to the myelin autoantigen may be used in accordance with the method of the present invention. For example, peptides may be synthesized comprising a defined amino acid sequence not based on the myelin antigen amino acid sequence, and yet are capable of down regulating an antigen specific immune response e.g. the peptide mimics a T cell epitope of the myelin autoantigen and causes down regulation of the immune response to the myelin autoantigen, or causes down regulation of the immune response for another reason, such as it is derived from a bystander antigen. Without being limited to any theory, it is believed that bystander antigens, which are also tissue specific (but are not the target of immune or autoimmune attack) may possess the ability to elicit suppressor T cells at the site of immune attack which may in turn result in down regulating the immune responses in the locality of the immune attack (e.g. afflicted "self" tissue in the case of autoimmune disease or nasal mucosa, skin and lung in the case of allergy). Bystander antigens include but are not limited to portions of the antigen or autoantigen which are not themselves the target of immune attack, and which possess suppressive activity at the site of immune attack. As used herein, the terms "myelin antigen" or "myelin autoantigen" includes bystander antigens which may possess suppressive activity at the site of myelin autoimmune attack.

In addition, any compound that mimics a peptide capable of down regulating an antigen specific immune response to a myelin autoantigen may be used in accordance with the invention (e.g. a peptidomimetic). Such a compound may not be composed entirely of subunits joined by peptide bonds, but joined by other linkages (e.g. thiolester bonds, reduced bond analogs, amide bond isosterases), providing that the non-peptide compound mimics a peptide capable of down regulating an antigen specific immune response to the antigen of interest as indicated by effective therapeutic/prophylactic treatment of symptoms. Peptidomimetics may be based on any of the peptides of the invention but may include for example, peptide bond

analogs (e.g. N-methyl amide bonds NH $C_{\alpha 2}$[-CO-NCH3-]$C_{\alpha 1}$) and reduced bond analogs (NH-$C_{\alpha 2}$[-CH_2-NH-]$C_{\alpha 1}$)) in place of one or more normal peptide bonds.

Once T-cell epitope-containing peptides have been identified, it is also possible to modify the structure of such peptides for use in accordance with the present invention for such purposes as increasing solubility (particularly desirable if the composition is to be injected), enhancing therapeutic or preventative efficacy (see discussion of peptide analogs having enhanced MHC binding below), or stability (e.g., shelf life ex vivo, and resistance to proteolytic degradation in vivo) or for ease of peptide synthesis. A modified peptide or peptide analog can be produced in which the amino acid sequence has been altered as compared to the native protein sequence from which it is derived, or as compared to the unmodified peptide from which the modified peptide is to be derived, such as by amino acid substitution, deletion, or addition, in order to modify immunogenicity, improve peptide solubility or increase the ease of peptide synthesis (e.g. automated peptide synthesis).

For example, a peptide can be modified so that it at least maintains, if not improves, the ability to down regulate the autoimmune response in MS (e.g. by inducing T cell non-responsiveness or reduced T cell responsiveness) and still retains the ability to bind MHC proteins. In this instance, critical binding residues for the T cell receptor can be determined using known techniques (e.g., substitution of each residue and determination of the presence or absence of T cell reactivity). Those residues shown to be essential to interact with the T cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance, diminish but not eliminate or not affect T cell activity. In addition, those amino acid residues which are not essential for T cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance, diminish but not eliminate or not affect T cell activity but does not eliminate binding to relevant MHC. Additionally, peptides of the invention can be modified by replacing an amino acid shown to be essential to interact with the MHC protein complex with another, preferably similar amino acid residue (conservative substitution) whose presence is shown to enhance, diminish but not eliminate or not affect T cell activity. In addition, amino acid residues which are not essential for interaction with the MHC protein complex but which still bind the MHC protein complex can be modified by being replaced by another amino acid whose incorporation may enhance, not affect, or diminish but not eliminate T cell reactivity. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine,

glutamic acid, or a methyl amino acid. In one example, WO 94/06828 describes substituted peptides in which essentially every amino acid residue may be substituted with a conservative amino acid, an amino acid not found in nature, or alanine, and yet the substituted peptide is still capable of down regulating an antigen specific immune response. In another example Karin et al. *J. Exp. Med.* **180**:2227-2237 (1994) describe studies using analogs of an immunodominant rat epitope of MBP having the amino acid sequence of 87-99 as shown in Fig. 14. The analogs were a series of 13 substituted peptides based on the 87-99 sequence that differed from the original 87-99 peptide by a single alanine substitution at each position along the 87-99 peptides. These studies were designed to show putative sites where the immunodominant peptide interacts with MHC and TCR in the Lewis rat as well as elucidate modified peptides having improved desired characteristics for potential therapeutic use. These studies showed that substituting the lysine (K) at position 91 of the 87-99 peptide with alanine (A) (see, 87-99[K>A] in Fig. 14) prevented and reversed EAE in Lewis rats. Based on this information one would expect that substituting alanine (A) for lysine (K) at the only lysine(K) present in peptides which contain the 87-99 sequence within their amino acid sequences i.e. MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, and MBP-2.6, all as shown in Fig. 2, would also result in increased T cell activity of the substituted peptides. For example MBP-2.1 substituted with alanine (A) for the lysine (K) at position 10 of the MBP-2.1 peptide (i.e. DENPVVHFFANIVTPRTPPPSQGK) may have enhanced T cell activity resulting in enhanced therapeutic properties as compared to the "parent" MBP-2.1 peptide.

In order to enhance stability and/or reactivity, peptides can also be modified to incorporate one or more polymorphisms in the amino acid sequence of the protein antigen resulting from natural allelic variation. Additionally, D-amino acids, non-natural amino acids or non-amino acid analogs can be substituted or added to produce a modified protein or peptide within the scope of this invention. Furthermore, peptides of the present invention can be modified using the polyethylene glycol (PEG) method of A. Sehon and co-workers (Wie et al. *supra*) to produce a protein or peptide conjugated with PEG. In addition, PEG can be added during chemical synthesis of a protein or peptide of the invention. Modifications of peptides or portions thereof can also include reduction/ alkylation (Tarr in: *Methods of Protein Microcharacterization*, J.E. Silver ed. Humana Press, Clifton, NJ, pp 155-194 (1986)); acylation (Tarr, *supra*); chemical coupling to an appropriate carrier (Mishell and Shiigi, eds., *Selected Methods in Cellular Immunology*, WH Freeman, San Francisco, CA (1980); U.S.

Patent 4,939,239; or mild formalin treatment (Marsh *International Archives of Allergy and Applied Immunology*, **41**:199-215 (1971)).

To facilitate purification and potentially increase solubility of proteins or peptides of the invention, it is possible to add reporter group(s) to the peptide backbone. For example, poly-histidine can be added to a peptide to purify the peptide on immobilized metal ion affinity chromatography (Hochuli, E. et al., *Bio/Technology*, **6**:1321-1325 (1988)). In addition, specific endoprotease cleavage sites can be introduced, if desired, between a reporter group and amino acid sequences of a peptide to facilitate isolation of peptides free of irrelevant sequences. To potentially aid proper antigen processing of T cell epitopes within a peptide, canonical protease sensitive sites can be recombinantly or synthetically engineered between regions, each comprising at least one T cell epitope. For example, charged amino acid pairs, such as KK or RR, can be introduced between regions within a peptide during recombinant construction of the peptide. The resulting peptide can be rendered sensitive to cathepsin and/or other trypsin-like enzymes cleavage to generate portions of the peptide containing one or more T cell epitopes.

Another example of a modification of peptides is substitution of cysteine residues preferably with serine, threonine, leucine or glutamic acid to minimize dimerization via disulfide linkages. In addition peptides may be modified to increase the solubility of a peptide for use in buffered aqueous solutions such as pharmaceutically acceptable carriers or diluents by adding functional groups to the peptide, terminal portions of the peptide, or by not including hydrophobic regions in the peptides. For example, to increase solubility, charged amino acids or charged amino acid pairs or triplets may be added to the carboxy terminus or amino terminus or both, of the peptide. Examples of charged amino acids include arginine (R), lysine (K), histidine (H), glutamic acid (E), and aspartic acid (D). For ease of peptide synthesis such as automated peptide synthesis, it may be desirable to delete or substitute amino acids which may make peptide synthesis more difficult or costly. For example, if the N-terminal or C terminal amino acid of the peptide is capable of cyclization, or may be subject to degradation either during or after peptide synthesis, such amino acids may be deleted, or substituted , or alternatively, one or more additional amino acid may be added to "block" the less desirable amino or carboxy terminal amino acid. Such added amino acids may either be derived from the native protein sequence or may be a non-native amino acid residue. Additional amino acids may be added to either the amino terminus, carboxy terminus or both, of a peptide for the purpose of increasing the peptide's T cell activity as defined above. Such

additional amino acids may be derived from the native protein sequence or be non-native amino acid residues.

Peptide compositions administered in accordance with the invention preferably comprise a sufficient percentage of the T cell epitopes of the myelin autoantigen (i.e. at least about 20%, more preferably about 30%, more preferably about 40%, and even more preferably about 60% or greater) of the total T cell reactivity to the myelin autoantigen in a population of individuals who respond to the autoantigen and who have multiple sclerosis (e.g. at least 10 individuals and more preferably at least 20 individuals) are included in the composition such that a therapeutic regimen of administration of the composition to an individual with MS in accordance with the invention, results in down regulation of the MS autoimmune response. To determine whether a peptide (preferred therapeutic candidate peptide) or a combination of candidate peptides are likely to contain a sufficient percentage of the total T cell reactivity of the myelin autoantigen to down regulate the MS autoimmune response in a substantial percentage of a population of individuals with MS, several analytical schemes may be used..

In accordance with one analytical scheme (using MBP as an example), T cell epitope-containing peptides are ranked according to the number of MBP microtiter culture lines responding to epitope-containing peptides and according to the number of MS patients responding to them. As the MBP -specific T cell frequency in the PBL of MS patients can be very low, it is often not possible to test all MBP peptides on every single MS patient. Therefore, the following means for determining the most useful therapeutic peptides is suitable and is further described in Example 1. PBL are isolated from blood and cultures are initiated in 96 well microtiter plates PBLs are purified from fresh peripheral blood specimens (approximately 75 cc) from patients with definite MS using a Ficoll density gradient. Microtiter cultures are initiated with 2×10^5 PBL per well and 10 ug/ml purified human spinal cord MBP in RPMI 1640 culture medium supplemented with 5% human AB serum, penicillin-streptomycin, and L-glutamine. Cultures are supplemented with IL2 (20 units/ml) and with IL4 (5 units/ml) beginning at day 6-7. After 11-13 days, the microtiter cultures are washed, resuspended in fresh media, and split into 12 fresh microtiter wells. Autologous frozen PBLs are added as antigen presenting cells at 5×10^4 PBL per well. Screening antigens are added in duplicate to the 12 replicate wells from each microtiter culture. Media is always used as a negative control, and purified human recombinant MBP at 10 ug/ml is used as a positive control. Each patient is also tested for reactivity with a maximum of 4 MBP peptides, each at a concentration of 10 uM. After 48 hours, the

assays are pulsed with 0.75 uCi of ^{3}H-thymidine, and harvested after a 6-16 hour pulse. Cultures are scored as positive for each peptide according to the following criteria: stimulation index greater than 3.0, change in cpm greater than or equal to 500, and standard error of the mean less than the change in CPM. In addition, for the purposes of analysis, cultures are scored as "peptide-positive" only if they respond to both MBP and to the peptide, and if they did not respond to more than one non-overlapping peptide. Groups of about 10-50 patients are preferably tested with each of the MBP peptides and each group of patients is tested with a maximum of four peptides. The peptides are then ranked in accordance with the following criteria: 1) the percent of MBP positive microtiter cultures in each group of patients (total MBP reactivity) which also scored positive for one of the MBP peptides; 2) the percent of MBP responder individuals in each group with at least one microtiter culture which scored positive for one of the MBP peptides, where an MBP responder individual is defined as a patient with at least one microtiter culture which scored positive for MBP. Individual peptide candidates are then selected if 1) they contain at least 5%, more preferably at least 10%, and most preferably at least 20% of the total MBP reactivity; and 2) reactivity to them is found in at least 20%, more preferably at least 30%, more preferably at least 40%, more preferably at least 50% and most preferably at least 60% of the MBP responder individuals. Optionally, additional criteria for ranking peptides may be considered such as the positivity index for a given peptide. The positivity index represents both the strength of the T cell response to a peptide (S.I.) and the frequency of a T cell response to a peptide in a population of individuals who respond to the myelin autoantigen. For example as shown in Fig. 15, the positivity index of 141-165 (MBP-4) is approximately 2500 which was calculated using data described in Example 1 herein. The mean S.I. of peptide MBP-4 per MBP responder patient was multiplied by the percent of individuals responding to MBP-4 in a population of patients responding to MBP.

Highly purified peptides substantially free from all other polypeptides and contaminants having a defined sequence of amino acid residues comprising at least one T cell epitope, used in therapeutic compositions of this invention, may be produced synthetically by chemical synthesis using standard techniques. Various methods of chemically synthesizing peptides are known in the art such as solid phase synthesis which has been fully or semi automated on commercially available peptide synthesizers. Synthetically produced peptides may then be purified, preferably to homogeneity, more particularly at least 90%, more preferably at least 95% and even more preferably at least 97% purity, substantially free from all other polypeptides and

contaminants using any number of techniques known in the literature for protein purification.

Synthetically produced peptides of the invention comprising up to approximately forty-five amino acid residues in length, and most preferably up to approximately thirty amino acid residues in length are particularly desirable as increases in length may result in difficulty in peptide synthesis. Peptides of longer length may be produced by recombinant DNA techniques as discussed below.

Peptides useful in the methods of the present invention may also be produced using recombinant DNA techniques in a host cell transformed with a nucleic acid sequence coding for such peptide. When produced by recombinant techniques, host cells transformed with nucleic acid encoding the desired peptide are cultured in a medium suitable for the cells and isolated peptides can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying peptides and proteins including ion-exchange chromatography, ultra filtration, electrophoresis or immunopurification with antibodies specific for the desired peptide. Peptides produced recombinantly may be isolated and purified, preferably to homogeneity, substantially free of cellular material, other polypeptides or culture medium for use in accordance with the methods described above for synthetically produced peptides.

In certain limited circumstances, peptides may also be produced by chemical or enzymatic cleavage of a highly purified full length or native protein of which the sites of chemical digest or enzymatic cleavage have been predetermined and the resulting digest is reproducible. Peptides having defined amino acid sequences can be highly purified and isolated substantially free of any other polypeptides or contaminants present in the enzymatic or chemical digest by any of the procedures described above for highly purified, and isolated synthetically or recombinantly produced peptides.

Additionally, peptides of the instant invention can be used in conjugates as disclosed, for example, in U.S. Patent 5,130,297 (Sharma et al.) where therapeutic agents are prepared using the formula X--MHC--peptide or MHC--peptide--X wherein X represents a functional moiety selected from a toxin and a labeling group; MHC is an effective portion of the MHC glycoprotein, said glycoprotein dissociated from the cell surface on which it normally resides; and "peptide" represents any of the peptides listed herein, more particularly MBP or MOG, even more particularly peptides shown in Figures 2 and 14.

Still further, preferred peptides of the invention comprise at least one T-cell epitope of the full length protein, more particularly MBP or MOG. The peptides may contain tandem repeats of a single epitope and/or more than one epitope.

In accordance with procedures described herein, for identifying peptides comprising T cell activity of MBP (see, discussion above as well as Example 1, below) preferred peptides derived from MBP and are candidates for therapeutic use comprise the following peptides: MBP-1, MBP-2, MBP-3, MBP-4 and MBP-5 all as shown in Fig. 2 or any portion thereof or any modification thereof. These peptides were tested for T cell activity as described in Example 1, and shown to comprise at least one T cell epitope as indicated by proportion of MBP positive microtiter cultures which also scored positive for one of the MBP peptides (Fig. 4a), and the detectable response for each of the preferred peptides in a significant percentage of MBP patients tested (Fig. 4b). MBP-4 (141-165) is the most reactive of the four peptides, accounting for 21% of the total MBP response, and detectable in 64% of the MBP-responder patients tested. MBP-4 surprisingly showed dramatically more reactivity than the combined reactivities of MBP 141-160 and MBP 151-170 as shown in Fig. 3. The present inventors are the first to identify this immunodominant peptide which appears to comprise multiple T cell epitopes. This novel peptide is particularly preferred for therapeutic use.

In addition, MBP-1.1, MBP-1.2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6 and MBP-3.1 as shown in Fig. 2 are also believed to be suitable candidate peptides for therapeutic use. These peptides are modified versions of MBP-1, MBP-2, and MBP-3 respectively and are expected to have similar T cell activity as that of their respective "parent" peptides. These peptides have been modified by amino acid deletion, addition or both in accordance with peptide modification techniques as described above, mainly for the purpose of ease of peptide synthesis.

Other peptides which have been shown to be immunodominant (i.e. have T cell activity of MBP), or have been derived from peptides known to have T cell activity of MBP, have been identified by the present inventors or by others working in this field (see, for example U.S.S. N. 08/328,224 filed on October 25; 1994; U.S.S.N. 08/241,246 filed on May 10, 1994; WO 93/21222; EP 0 304 279, WO 91/15225; Ota et al, *Letters to Nature*, **346**:183-187 (1990); Wucherpfennig et al., *J. Exp. Med.*, **170**:279-290 (1994); Martin et al., *J. Immunol.*, (1990) **145**:540-548); Karin et al., *J. Exp. Med.*, **180**:2227-2237 (1994)). Such peptides may also be suitable for therapeutic use in compositions and methods of this invention, particularly when combined with the preferred peptide candidates described above. Such peptides

include but are not limited to, all or a portion of the following peptides having residue numbers which correspond to the amino acid residues of human MBP protein shown in Fig. 1 and having individual amino acid sequences which are shown in Fig. 14: 13-25, 31-50, 61-80, 82-92, 82-96, 82-97, 82-98, 82-100, 82-100 [100 P>Y], 83-100, 83-101, 84-97, 84-100, 84-100 , 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, even more preferably comprise the following peptides: 13-25, 87-99, 87-99 [91K>A], 82-100, 82-100 [100P>Y], all as shown in Fig. 14. Preferred portions of these peptides or preferred modifications would preferably have similar or greater T cell activity in the same or greater percentage of patients tested and/or have similar or greater therapeutic effectiveness in the methods of the invention as that of the "parent" peptide from which the modified peptide was derived.

One aspect of the present invention provides therapeutic compositions comprising at least one peptide derived from a myelin antigen having T cell activity, or a combination of peptides derived from a myelin antigen, each peptide having T cell activity, and a pharmaceutically acceptable carrier or diluent. Preferred therapeutic compositions comprise a sufficient percentage of the T cell activity of the myelin autoantigen such when administered to an MS patient in a therapeutic regimen, preferably in a non-immunogenic form, are capable of down regulating the myelin autoantigen specific immune response in a population of humans subject to such antigen specific immune response. As used herein, "down regulation" includes, but is not limited to, preventing initial onset of disease symptoms, reducing the disease symptoms of multiple sclerosis caused by the antigen specific immune response to MBP or other myelin autoantigen, more particularly, reduction, reversal, non-progression or alleviation of symptoms. Non-progression may be characterized by, but is not limited to (a) shorter periods of active disease or exacerbation, (b) less severe symptoms or disability, (c) delay in disease progression wherein the baseline health does not go down as quickly, (d) extension or elongation of the lengths of time between periods of active disease or exacerbation (e.g. longer periods of remission), (e) fewer relapses or exacerbations and/or (f) slowing or arresting progression of MRI-detected lesion load. The Expanded Disability Status Scale (EDSS) scoring system and Neurological Rating Scale are other assessment tools used by those skilled in the relevant art. As used herein, "advanced stage" is any point beyond clear clinical signs of overt disease whether the disease is relapsing-remitting MS, chronic progressive MS, benign MS or primary progressive MS. Further to this definition, "advanced stage" may comprise acute phase(s), remission(s) and exacerbation(s). As used herein

the term "acute phase" shall mean ongoing attack, acute disease, active disease, and exacerbation and are used interchangeably. As they are used herein, these terms generally refer to the state where the subject suffering from the disease (whether diagnosed or not) presents with active symptoms or signs commonly understood by those skilled in the art as associated with the specific immune response characteristic of multiple sclerosis. A "relapse" is understood to mean an acute phase which follows a remission. The term exacerbation, if used in the appropriate in context, can also be interpreted to mean new and worsening symptoms or signs. "Symptoms" are those indicia of disease of which the patient complains. "Signs" are those indicia observed or measured by the diagnostician. However, the terms symptoms and signs shall be used interchangeably unless otherwise indicated.

Therapeutic compositions of the invention preferably comprise at least one T cell epitope-containing peptide or modified peptide or peptide analog and a pharmaceutically acceptable carrier or diluent. Such compositions may preferably comprise a MBP peptide selected from the following group of peptides: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4 and MBP-5, more preferably an MBP peptide is selected from MBP-1.1, MBP-2.1, MBP-4 and MBP-5, and even more preferably an MBP peptide is MBP-4.

Compositions of the invention may comprise at least two peptides (e.g. a physical mixture of at least two peptides), each peptide having T cell activity and preferably comprising at least one T cell epitope of a myelin autoantigen such as MBP. Such compositions can be administered in the form of a therapeutic composition with a pharmaceutically acceptable carrier or diluent. A therapeutically effective amount of one or more of such compositions can be administered simultaneously or sequentially to an individual suffering from MS. Preferred compositions comprise at least one and preferably comprise at least two peptides selected from the following group of peptides: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, and MBP-5 all as shown in Fig 2, and even more preferably are selected from the following group of peptides: MBP-1.1, MBP-1.2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3.1, MBP-4, and MBP-5 and most preferably is selected from the following group of peptides: MBP-1.1, MBP-2.1, MBP-4 and MBP-5. Additionally, compositions of the invention may further comprise peptides derived from MBP having residue numbers which correspond to the amino acid residues of the human MBP protein shown in Fig. 1 and have individual amino acid

sequences as shown in Fig. 14: 13-25, 31-50, 61-80, 82-92, 82-96, 82-97, 82-98, 82-100, 82-100 [100P>Y], 83-100, 83-101, 84-97, 84-100, 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111,-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, and even more preferably comprise the following peptides: 13-25, 87-99, 87-99 [91K>A], 82-100, 82-100 [100P>Y]. Preferably, compositions of the invention comprise at least two peptides wherein at least one peptide is MBP-4 and at least one peptide is selected from the following group of peptides: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, and MBP-5 all as shown in Fig. 2, and may further comprise at least one of the following peptides derived from MBP as shown in Fig. 14: 13-25, 31-50, 61-80, 82-92, 82-96, 82-97, 82-98, 82-100, 82-100 [100P>Y], 83-100, 83-101, 84-97, 84-100, 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111,-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, and even more preferably comprise at least one of the following peptides: 13-25, 87-99, 87-99 [91K>A], 82-100, 82-100 [100P>Y].

Preferred compositions of the invention comprise the following peptides:

MBP-1, MBP-2, MBP-3, and MBP-4, and MBP-5;

MBP-1.1, MBP-2.1, MBP-3, MBP-4, and MBP-5

MBP-1.1, MBP-2, MBP-4, and MBP-5;

MBP-1, MBP-2.1, MBP-4, and MBP-5;

MBP-1, MBP-2, MBP-4, and MBP-5;

MBP-1.1, MBP-2.1, MBP-4, and MBP-5;

MBP-1.1, MBP-2.1, and MBP-4;

MBP-1, MBP-2.1, and MBP-4;

MBP-1.1, MBP-2, and MBP-4;

MBP-1.1, MBP-2.1, and MBP-5;

MBP-1.1, MBP-2.1, and MBP-3;

MBP-1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-1.1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-4, and a selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-4, and a peptide selected from the group consisting of: MBP-1, or MBP-1.1;

MBP-1.1, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;

MBP-1.1, and MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.
MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;
MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.
MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;.
MBP-1.1, MBP-3, MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

The present invention also contemplates a treatment regimen comprising administering combinations of therapeutically effective peptides as a single treatment episode. Such combinations of peptides may be administered simultaneously or sequentially as therapeutic compositions comprising only one peptide or comprising several peptides. Such a treatment regimen may not necessarily be a physical mixture of more than one peptide, but does comprise a combination of peptides administered simultaneously or sequentially as a single treatment episode. Preferred combinations of peptides (in the form of one or more compositions each comprising one or more peptides) which can be administered simultaneously or sequentially as a single treatment episode include the following combinations of peptides:
MBP-1, MBP-2, MBP-3, and MBP-4, and MBP-5;
MBP-1.1, MBP-2.1, MBP-3, MBP-4, and MBP-5
MBP-1.1, MBP-2, MBP-4, and MBP-5;
MBP-1, MBP-2.1, MBP-4, and MBP-5;
MBP-1, MBP-2, MBP-4, and MBP-5;
MBP-1.1, MBP-2.1, MBP-4, and MBP-5;
MBP-1.1, MBP-2.1, and MBP-4;
MBP-1, MBP-2.1, and MBP-4;
MBP-1.1, MBP-2, and MBP-4;
MBP-1.1, MBP-2.1, and MBP-5;
MBP-1.1, MBP-2.1, and MBP-3;
MBP-1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;
MBP-1.1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-4, and a selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-4, and a peptide selected from the group consisting of: MBP-1, or MBP-1.1;

MBP-1.1, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;

MBP-1.1, and MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;

MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;.

MBP-1.1, MBP-3, MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

In addition, preferred compositions and preferred combinations of MBP peptides which can be administered simultaneously and/or sequentially may include any of the above compositions and combinations and in addition, may also comprise at least one T cell epitope-containing peptide derived from myelin oligodendrocyte protein (MOG), another protein which is believed to be one of the autoantigens involved in multiple sclerosis (see, Lebar, et al., *J. Immunol.* (1976) **116**:1439-1446; Lebar et al., *J.Exp. Immunol* (1986) 66:423-443; Linington and Lassman, *J. Neuroimmunol.* (1987) **17**:61-69; Lassman et al., *Acta Neuorpathol.* (Berl) (1988) **75**:566-576; and Sun et al., *J. Immunol.* (1991) **146**:1490-1495) Peptides which may comprise T cell epitopes derived from MOG, are disclosed in USSN 08/116,824 filed September 3, 1993 and USSN. 08/300,811 filed September 1, 1994, (incorporated herein by reference), and which are expected to be effective in the treatment of multiple sclerosis when prepared and/or administered in conjunction with the above-described compositions and combinations of MBP T cell epitope-containing peptides according to the instant invention are:

Human MOG 1-13	GQFRVIGPRHPIR
Human MOG 103-115	HSYQEEAAMELKV
Human MOG 1-121	GQFRVIGPRHPIRALVGDEV
	ELPCRTSPGKNATGMEVGWY
	RPPFSRVVHLYRNGKDQDGD
	QAPEYRGRTELLKDAIGEGK
	VTLRIRNVRFSDEGGFTCFF

RDHSYQEEAAMELKVEDPFYW

Additional epitope-containing peptides of MOG have been identified by the present inventors using an experimental analysis similar to that described above and in Example 1 (data not shown) such peptides include:

Human MOG 1-20	GQFRVIGPRHPIRALVGDEV;
Human MOG 11-30	PIRALVGDEVELPCRISPGK;
Human MOG 21-40	ELPCRISPGKNATGMEVGWY;
Human MOG 31-50	NATGMEVGWYRPPFSRVVHL;
Human MOG 141-160	TVGLVFLCLQYRLRGKLRAE;
Human MOG 151-170	YRLRGKLRAEIENLHRTFDP;
Human MOG 161-180	IENLHRTFDPHFLRVPCWKI; and
Human MOG 199-218	YNWLHRRLAGQFLEELRNPF.

The present invention further contemplates modifications or analogs (discussed earlier) of the above T cell epitope-containing MOG peptides which retain similar or greater T cell activity as the parent peptide from which the modification or analog is derived.

It is believed that compositions comprising T cell epitope-containing peptides of both MOG and MBP, modifications thereof, analogs thereof, or peptidomimetics based thereon, and combinations of such peptides which can be administered simultaneously or sequentially have the advantage of maximizing the down-regulating effect on both MBP and MOG specific T cells participating in the autoimmune response in MS. In this manner a range of T cells which may participate in the autoimmune response to either MBP or MOG resulting in the clinical manifestations of MS (demyelation) may be targeted for down regulation thereby enhancing the therapeutic effect of the compounds and compositions of the invention. Likewise, T cell epitope containing peptides derived from other myelin antigens believed to be autoantigens in MS (e.g. proteolipid protein (PLP) and myelin associated glycoprotein (MAG)) may also be suitable in compositions and methods of the invention.

A therapeutic/prophylactic treatment regimen in accordance with the invention (which results in reversal of , prevention of, or delay in, the onset of disease symptoms caused by an offending autoantigen or results in reduction, non-progression, or alleviation of symptoms caused by an offending autoantigen i.e. down regulation of an autoantigen specific immune response) comprises administration, in non-immunogenic form of a therapeutic composition of the invention comprising at least one isolated peptide derived from an autoantigen responsible for the disease condition being treated (e.g. MBP, MOG, PLP, MAG). While not intending to be limited to any theory, it is believed that administration of a therapeutic composition of

the invention may: a) cause T cell non responsiveness of appropriate T cell subpopulations such that they become unresponsive to the offending antigen and do not participate in stimulating an immune response upon exposure to the offending protein antigen (e.g. via anergy or apoptosis); b) modify the lymphokine secretion profile as compared with exposure to the offending autoantigen; c) cause T cell subpopulations which normally participate in the response to the offending antigen to be drawn away from the sites of normal exposure towards the sites of administration of the composition (this redistribution of T cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the offending antigen, resulting in diminution or reversal in symptoms); d) cause induction of T suppressor cells or e) cause induction of suppressor cells via a bystander antigen.

Highly purified and isolated peptides produced as discussed above may be formulated into therapeutic compositions of the invention suitable for human therapy. If a therapeutic composition of the invention is to be administered by injection (e.g. subcutaneous injection, intravenous injection), then it is preferable that the highly purified peptide be soluble in an aqueous solution at a pharmaceutically acceptable pH (i.e. pH range of about 4-9) such that the composition is fluid and easy syringability exists. The composition also preferably includes a pharmaceutically acceptable carrier. As used herein "pharmaceutically acceptable carrier" includes any and all excipients, solvents, dispersion media, coatings, antibacterial and antifungal agents, toxicity agents, buffering agents, absorption delaying or enhancing agents, surfactants, and micelle forming agents, lipids, liposomes, and liquid complex forming agents, stabilizing agents, and the like. The use of such media and agents for pharmaceutically active substance is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

As discussed above, therapeutic compositions of the invention suitable for injectable use are preferably sterile aqueous solutions prepared by incorporating active compound (i.e., one or more highly purified and isolated peptides as described above) in the required amount in an appropriate vehicle with one or a combination of ingredients enumerated above and below, as required, followed by filtered sterilization. Preferred pharmaceutically acceptable carriers include at least one excipient such as sterile water, sodium phosphate, mannitol, sorbitol, or sodium chloride or any combination thereof. Other pharmaceutically acceptable carriers

which may be suitable include solvents or dispersion medium containing, for example, water, ethanol, polyol (for example glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained for example by the use of coating such as lecithin, by the maintenance of the required particle size in the case of dispersions and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thirmerosol and the like. Prolonged absorption of the injectable compositions can be brought about by including in the composition, an agent which delays absorption, for example, aluminum monostearate and gelatin.

Preferable therapeutic compositions of the invention should be sterile, stable under conditions of manufacture, storage, distribution and use and should be preserved against the contaminating action of microorganisms such as bacteria and fungi. A preferred means for manufacturing a therapeutic composition which maintains the integrity of the composition (i.e. prevent contamination, prolong storage, etc.) is to prepare the formulation of peptide and pharmaceutically acceptable carrier(s) such that the composition may be in the form of a lyophilized powder which is reconstituted in a pharmaceutically acceptable carrier, such as sterile water, just prior to use. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying, freeze-drying or spin drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

As discussed above, a therapeutic composition of the invention may comprise more than one isolated peptide. A therapeutic composition comprising a multipeptide formulation suitable for pharmaceutical administration to humans may be desirable for administration of several active peptides. The multipeptide formulation includes at least two or more isolated peptides having a defined amino acid sequence and is capable of down regulating an antigen specific immune response. Any of the compositions described earlier which comprise at least two peptides may be suitable as a multipeptide formulation. As discussed earlier, highly preferred peptides suitable for use in a multipeptide formulation include at least two of the following peptides: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1 MBP-4 and MBP-5. Special considerations when preparing a multipeptide formulation include maintaining the solubility, and stability of all peptides in the formulation in an aqueous solution at a physiologically acceptable pH. This requires choosing one or more pharmaceutically acceptable

solvents and excipients which are compatible with all the peptides in the multipeptide formulation. For example, suitable excipients include sterile water, sodium phosphate, mannitol or both sodium phosphate and mannitol. An additional consideration in a multipeptide formulation is the prevention of dimerization of the peptides if necessary.

Administration of the therapeutic compositions as described above to an individual, in a non-immunogenic form, can be carried out using known procedures at dosages and for periods of time effective to cause down regulation of the MBP antigen specific immune response of the individual being treated for MS. Down regulation of an antigen specific immune response to an antigen associated with a disease condition in humans may be determined clinically whenever possible, or may be determined subjectively (i.e. the patient feels as if some or all of the symptoms related to the disease condition being treated have been alleviated).

Effective amounts of the therapeutic compositions of the invention may vary according to factors such as the degree of sensitivity of the individual to the antigen, the age, sex, and weight of the individual, and the ability of peptide to cause down regulation of the antigen specific immune response in the individual. A therapeutic composition of the invention may be administered in non-immunogenic form, in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, sublingual, inhalation, transdermal application, rectal administration, or any combination of routes of administration designed to enhance therapeutic effectiveness, or any other route of administration known in the art for administering therapeutic agents. It may be desirable to administer simultaneously or sequentially a therapeutically effective amount of one or more of the therapeutic compositions of the invention to an individual. Each of such compositions for administration simultaneously or sequentially, may comprise only one peptide or may comprise a multipeptide formulation as described above.

To administer peptide or peptide composition by other than parenteral administration, it may be necessary to coat the peptide with, or co-administer the peptide with, a material to prevent its inactivation. For example, a peptide composition may be co-administered with enzyme inhibitors or in liposomes. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan et al., (1984) *J. Neuroimmunol.* 7:27). When a peptide is suitably protected, as described above, the peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The

peptide composition and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the composition and preparations may, of course, be varied and may conveniently be between about 5 to 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit contains between from about 10 μg to about 200 mg of active compound. The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservative, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

For injection, (subcutaneous, I.V., I.M., intraperitoneal) of one or more therapeutic compositions of the invention, preferably about 1 μg- 3 mg and more preferably from about 20 μg-1.5 mg, and even more preferably about 50 μg- 750 μg, and even more preferably about 75 μg to about 750 μg, of each active component (peptide) per dosage unit may be administered. Depending upon the regimen as described below, doses as high as 1500 μg or more may be used. It is especially advantageous to formulate parenteral compositions in unit dosage form for ease of administration and uniformity of dosage. "Unit dosage" form as used herein refers to physically discrete units suited as unitary dosages for human subjects to be treated; each unit containing a predetermined quantity of active compound calculated to

produce the desired therapeutic effect in association with the desired pharmaceutical carrier. The specification for the novel unit dosage forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of human subjects.

Dosage regimen may be adjusted to provide the optimum prophylactic or therapeutic response. For example, several divided doses may be administered over the course of days, weeks, months or years, or the dose may be proportionally increased or reduced with each subsequent injection as indicated by the exigencies of the therapeutic situation. In one preferred therapeutic regimen, subcutaneous injections of therapeutic compositions are given once a day during an acute phase and once every other day during remission for the lifetime of the individual suffering from the disease. Alternatives would include, weekly, monthly or other periodic injections. The dosage may remain constant for each injection or may increase or decrease with each subsequent injection. A continual, lifetime treatment program may be most desirable. In the alternative, a booster injection may be administered at intervals of about three months to about one year after an initial treatment period and may involve only a single injection or may involve another series of injections similar to that of the initial treatment.

Because of the highly individual immune system response of individuals suffering from either relapsing remitting MS, primary progressive MS, benign MS, and chronic progressive MS, careful and varied dosage regimens will need to be developed. First, an intake evaluation of the presenting patient must be completed. A complete exam is conducted looking for, among other things, impaired vision. nystagamus, dysarthria, decreased perception of vibration and position sense, ataxia and intention tremor, weakness or paralysis of one or more limbs, spasticity and bladder problems. Those skilled in the art would use accepted tools such as EDSS, Neurological Rating Scale or other similar tools known in the art as well as the previously discussed indicia of disease state to determine a baseline from which any change, including disability progression, but preferably down regulation, could be measured. Although there is no typical MS acute or remitting phase, certain patterns have emerged which would guide the experienced practitioner. As mentioned above, the frequency of flare-ups or acute stages is greatest during the first 3 to 4 years of disease, but a first attack, may not be followed by another observable attack for 10 to 20 years (although lesion load detectable only by MRI might give a different clinical picture) . During typical episodes, symptoms worsen over a period of a few days to 2

to 3 weeks and then remit. Recovery is usually rapid over a period of weeks, although at times it may extend over several months.

MS is a chronic disease and continual therapy is contemplated. On going, long term treatment may be the most effective treatment to stem the progression of disease and disability. A dosage regimen of every other day, at least once a week or once a month may be appropriate. Reasonable modifications are within the abilities of one skilled in the art. Intervention at onset of an attack is considered an important component in effective treatment.

For a patient presenting in the acute stage, the severity of attack must evaluated. An initial injection may be given at the onset of the acute stage. The obvious benefit would be to initiate treatment as early into the acute phase as possible. If desired, this therapy could be combined with simultaneous treatment with β-interferon, steroids and/or other therapies especially those designed to decrease inflammation. The patient would be observed daily after the first treatment, preferably by injection. It is suggested that additional treatments be given every day, or at least every third day during the acute phase. As with any medication, the treating physician should modify the dosage based upon clinical changes which indicate the need for modification. Any such discretion is within the scope of those skilled in the art using the suggested dosage schedule and amounts as guidelines. The dosage range from about 75 to about 750 μg per dose gives a great deal of latitude (and not an unreasonable amount) to the treating physician. Options include but are not limited to daily treatments during acute phase after which the course of treatment for remission set out above is followed. This by no means excludes the possibility of more or less frequent dosing if the treating physician determine intervention is indicated.

The present invention discloses that "advanced stage" multiple sclerosis can be treated in accordance with compositions and methods of the invention. Compositions comprising at least one peptide having T cell activity and derived from a myelin autoantigen is suitable for treating advanced stage MS. Compositions of MBP peptides, MOG peptides and combinations thereof described earlier are suitable for treating advanced stage MS. As described above these peptides include but are not limited to: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, MBP-5, 13-25, 31-50, 61-80, 82-92, 82-96, 82-97, 82-98, 82-100, 82-100 [100P>Y], 83-100, 83-101, 84-97, 84-100, 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111,-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, and preferably MBP-1.1,

MBP-2.1, MBP-4, MBP-5, 13-25, 87-99, 87-99 [91K>A], 82-100, 82-100 [100P>Y] as well as peptides derived from human MOG:

Human MOG 1-13	GQFRVIGPRHPIR
Human MOG 103-115	HSYQEEAAMELKV
Human MOG 1-121	GQFRVIGPRHPIRALVGDEV
	ELPCRTSPGKNATGMEVGWY
	RPPFSRVVHLYRNGKDQDGD
	QAPEYRGRTELLKDAIGEGK
	VTLRIRNVRFSDEGGFTCFF
	RDHSYQEEAAMELKVEDPFYW
Human MOG 1-20	GQFRVIGPRHPIRALVGDEV;
Human MOG 11-30	PIRALVGDEVELPCRISPGK;
Human MOG 21-40	ELPCRISPGKNATGMEVGWY;
Human MOG 31-50	NATGMEVGWYRPPFSRVVHL;
Human MOG 141-160	TVGLVFLCLQYRLRGKLRAE;
Human MOG 151-170	YRLRGKLRAEIENLHRTFDP;
Human MOG 161-180	IENLHRTFDPHFLRVPCWKI; and
Human MOG 199-218	YNWLHRRLAGQFLEELRNPF.

The present inventors have are the first to make the surprising discovery that intervention in the midst of an ongoing attack, actually improves the condition of a subject treated accordingly to the method of the instant invention. At a minimum, such intervention does not worsen the condition of the subject. Further, applicants show that intervention during remission down regulates the immune response and the condition does not become activated as a result of any such intervention. Thus, not only may the intervention cause improvement but appears to be entirely safe when administered according to the method of the instant invention. Using the EAE model as described above, applicants demonstrate that repeated intravenous administration of the immunodominant MBP peptide Ac1-11 successfully treats EAE induced with the entire MBP protein. Once successful treatment (indicative of T cell reactivity) is shown with a "native peptide" (unsubstituted peptide derived from the native sequence in accordance with procedures described herein for identifying T cell epitope-containing peptides derived from a protein autoantigen) suitable modified peptides may be identified by comparing binding affinities of the modified peptide with the native peptide. A modified peptide which has a binding affinity lower than the native peptide would be a suitable candidate peptide for a therapeutic composition of the instant invention. Assays for determining binding affinities can be identified according to the teachings of U.S.S.N. 08/300,811 filed September 1, 1994 incorporated herein by reference. For example, Ac1-11 therapy was found to be successful. Ac1-11 therapy was then compared to therapy with Ac1-11[4Y], an Ac1-11 analog which binds to $A\alpha^{u}A\beta^{u}$ with higher affinity and greater stability (Wraith, D.C. et al., *supra* and Fairchild, P.J. et al. *supra*). Interestingly, applicants found that Ac1-11[4Y] is effective at a 100-fold lower dose than Ac1-11, and that its therapeutic effect is longer lasting indicating that selected modified peptides would be also

suitable in a therapeutic composition . Further, applicants show that the particular peptide tested Ac1-11[4Y] forms stable peptide-MHC complexes *in vivo* in the treated mice. This indicates that modified peptides which form stable peptide-MHC complexes *in vivo* are highly likely to be suitable peptides in the treatment of humans as their potency may be greatly increase with conservative amino acid substitutions (see also, Karin et al., *J. Exp. Med.*, **180**:2227-2237 (1994)). Applicants also discovered that the MBP peptide analog Ac1-11[4Y] reversed ongoing paralysis when administered during the acute phase of EAE, and that it prevented relapses when administered during remission. This indicates that selected modified peptides and selected peptide analogs may reverse active symptoms and prevent relapses in humans suffering from MS.

In still another model for the course of human disease. the applicant initiated intravenous peptide therapy after the onset of EAE and during an acute phase. Quite surprisingly, this therapy reversed ongoing disease when administered during the acute phase. This finding is the first of its kind. In past experiments done by others, peptide therapy was initiated at a time when the majority of the mice in the treatment group had not yet shown signs of EAE, prior to immunization or near the onset of disease, at the latest. For example, in Gaur et al., *supra*, a group of 13 mice were subjected to a disease provoking regimen of MBP. When only one of the 13 mice showed minimal signs of disease, i.e., one mouse had a clinical score of 1 (see Example 2 for a discussion of clinical scoring.in mice), seven of the mice were injected intraperitoneally with MBP peptide mixture. The remaining six mice, one of which was the animal showing the signs of disease received no additional treatment. In this case, for those mice without clinical symptoms of disease, Gaur et al. is unable to draw any meaningful conclusion about their data. The presence of a low clinical score of 1 in one mouse cannot be interpreted to indicate active disease in any of the other mice. Accordingly, Gaur et al. discloses treatment before onset of the disease, and most definitely, before clinical signs of disease. By contrast, in order to be useful in a clinical setting for treatment of humans with MS, a therapy would need to be effective even if initiated well after the onset of the disease process. As a model of appropriate human therapy, applicants administered peptide therapy to mice with 250 nmol Ac1-11[4Y] initiated after the onset of the acute phase of EAE. Figure 5 shows the effect of this therapy. The therapy was initiated individually in each mouse as it exhibited initial signs of EAE. Mice were assigned as they developed EAE to the Ac1-11[4Y] peptide therapy group and to each of the three control groups (no treatment, PBS, or the $A\alpha^{u}A\beta^{u}$ -binding peptide OVA 323-337). Figure 9 shows that

therapy of ongoing EAE with Ac1-11[4Y] leads to dramatic improvement in disease scores apparent within 48 hours of the initial injection of peptide. EAE nearly totally resolved in these treated mice by the fourth day after onset, and the effect of an 18 day course of peptide therapy was prolonged.

In still another surprising development, applicants discovered that intravenous peptide therapy initiated during the remission phase of EAE prevents relapses. Again, using the EAE as a model for human disease, as mentioned above, (PLJ x SJL)F1 mice develop relapsing EAE upon immunization with MBP (Fritz, R.B., et al., *J. Immunol.*, 1983, **130**:1024). EAE usually develops between 9 and 16 days following immunization, and the acute phase of disease in this model lasts from approximately 3 to 20 days. Twenty percent or more of the mice may die or become moribund during the acute phase, depending on disease severity in the experiment. Most of the acute phase survivors then enter a brief remission phase, displaying mild or no clinical signs of disease for several days. In some experiments, mice all enter the remission phase at about the same time (illustrated in Figure 5), but in most cases the length of the acute phase of disease is more variable. The remission phase is followed by a first relapse phase, which is generally equal in severity to the acute phase of disease. Subsequently, surviving mice develop residual chronic paralysis, which persists until termination of the experiment.

In the mouse model, the peptide, Ac1-11[4Y], is a remarkably effective therapy when initiated during the remission phase of EAE. In order to examine the capacity of Ac1-11[4Y] to prevent EAE relapses, mice were followed throughout their initial episodes of paralysis which lasted from 3-19 days. As surviving mice entered the remission period individually (defined as a reduction in clinical score to 1 or 0 for at least two days), they were alternately assigned to two groups and intravenous therapy was initiated with 25 nmoles of Ac1-11[4Y] or with the control PBS. Mice were treated individually beginning on their second day of remission. Figure 10 shows that intravenous treatment with Ac1-11[4Y] beginning during the remission phase of EAE dramatically reduced the incidence and severity of relapse, and indicates that intravenous peptide therapy can be effective even when initiated late in the disease course.

The mechanism of intravenous peptide therapy has not been fully elucidated, although it has previously been suggested in a number of other antigen systems administration of an antigenic peptide intravenously prior to immunization induces antigen-specific non-responsiveness of the immune system. As further indication of the viability of a therapeutic administered to MS patients, applicants administered I.V.

encephalitogenic peptide or peptide analog and found that it reduced *in vitro* lymph node proliferation and IL2 production. Mice were injected intravenously with 250 nmoles of MBP Ac1-11 ten and five days prior to immunization with MBP protein. Figure 11a shows the lymph node proliferative response from these treated mice measured on day 10 following immunization. Intravenous pretreatment with the immunodominant MBP peptide Ac1-11 without adjuvant prior to immunization reduces the subsequent *in vitro* proliferative T cell response to MBP Ac1-11. Similar results were obtained when mice were pretreated with intravenous injections of the MBP peptide analog Ac1-11[4Y] (data not shown). Moreover, lymph node IL2 production in response to MBP Ac1-11 was prevented by intravenous pretreatment with Ac1-11[4Y] (Figure 11b). The results confirm that intravenous pretreatment with MBP Ac1-11 or Ac1-11[4Y] induces T cell non-responsiveness to MBP Ac1-11.

Thus, applicants have examined the efficacy of therapy with intravenous MBP peptides and peptide analogs in treating relapsing EAE, and have made a number of unique observations. First, and perhaps most important, treatment of an autoimmune disease with a single autoantigenic peptide can be effective even when therapy is initiated after the onset of disease signs. Specific disease signs used for scoring the mice are discussed in the Examples herein. A Mean Clinical Scores of a level 2 or above was defined as advanced stage EAE. The results indicate that intravenous MBP peptides interfere with the encephalitogenic activity of autoaggressive primed cells, even after the blood brain barrier has been disrupted and lymphocytic infiltration of the central nervous system has occurred. At no time were the peptides observed to activate, aggravate or exacerbate ongoing disease, even temporarily. EAE treated after the onset of the acute phase was reversed within a few days of initiation of therapy, even though initially the treated mice were severely affected. Histological examination confirmed that a clinical response to peptide therapy correlated with a marked reduction in brain and spinal cord lymphocytic infiltrates. Also, as discussed herein I.V. administration of MBP Ac1-11[4Y] was effective even beginning as late as thirty days after immunization with MBP, in the remission phase of EAE. Thus, initiation of treatment during an apparently quiescent phase of the disease once again did not lead to exacerbations, and, moreover, completely prevented all but the mildest of relapses. This result is particularly interesting since there is evidence that recognition of a variety of MBP epitopes occurs late in the immune response to MBP, and it has been proposed that these epitopes may contribute to disease (Lehmann, P.V., et al., *Nature*, 1992, **358**:155). The present invention suggest that the administration of peptide very early in the disease course might prevent the

development of a late immune response to such epitopes. However, it is notable that a single MBP peptide analog effectively prevented relapses when administered after resolution of the severe acute phase of EAE, when the immune response to MBP and possibly to other myelin antigens may have already progressed.

In the practice of the instant invention, the selection of a potent therapeutic is preferred and is described earlier herein. Additionally, the present inventors theorized that one criteria for the best peptide candidates would be those with T cell reactivity and higher MHC binding affinity than the native peptide which form stable complexes with class II MHC *in vivo*. In particular, applicants examined the MBP peptide analog which forms stable complexes with class II MHC *in vivo* is more effective than Ac1-11 in treating EAE. It has been shown previously that the MBP peptide analog Ac1-11[4Y] binds to $A\alpha^{u}A\beta^{u}$ with a higher affinity, and that complexes formed *in vitro* between Ac1-11[4Y] and $A\alpha^{u}A\beta^{u}$ are more stable than those formed between Ac1-11 and $A\alpha^{u}A\beta^{u}$. Ac1-11[4Y] also has been shown to prevent EAE when a large dose is inhaled prior to immunization (Metzler, B. et al., *supra*). The instant invention discloses that a modified MBP peptide analog Ac1-11[4Y] is at least 100-fold more potent as a therapeutic agent than MBP Ac1-11, and requires a less frequent dosing schedule. This increased potency results in a therapeutic which is both more practical and more efficacious. Applicants have observed that intravenous administration of Ac1-11[4Y] (but not Ac1-11) results in the formation of stable peptide-MHC complexes *in vivo*. The improved potency of Ac1-11[4Y] is believed to be related this formation of stable peptide-MHC complexes. These complexes are detectable on spleen cells using an MBP Ac1-11-specific hybridoma for up to ten hours after injection. Accordingly, it is believed that formation of stable peptide-MHC complexes may be one of the hallmarks of such a potent therapeutic peptide. Surprisingly, the *in vivo* therapeutic effect of Ac1-11[4Y] was present long after peptide-MHC complexes became undetectable on spleen cells (see Figure 6a and Figure 9). It is likely that the peptide-MHC complexes form antigen-specific functional therapeutic units which exert a long-lasting effect on encephalitogenic T cells, persistent after the complexes are no longer detectable *in vivo*. It is not known whether such complexes exert their effect peripherally, or in the central nervous system. If they operate in the central nervous system, the complexes may be formed there, or cells bearing the complexes may migrate across the blood-brain barrier.

The present invention further provides methods for treating multiple sclerosis comprising administering a therapeutically effective amount of at least one peptide of

MBP having T cell activity in a treatment regimen which includes a therapeutically effective amount of IFN-β.

As a result of the work described herein it has been discovered that a combination of at least one peptide having T cell activity derived from a myelin antigens (e.g. MBP), and IFN-β, when administered in a therapeutic regimen, has a synergistic effect (Fig. 12c) which surprisingly diminishes the clinical symptoms of EAE in mice to a far greater extent than the effect of each on mitigating the symptoms of EAE when administered alone (Figs. 12a-b), and which is greater than what one would expect for a merely additive effect of the peptide plus IFN-β.

As EAE serves as a mouse model of human MS and is induced by various myelin antigens (e.g. PLP, MBP, MOG), it is expected that a similar effect would also be seen in humans. Therefore, the present invention provides a method of treating individuals who have multiple sclerosis or are susceptible to developing multiple sclerosis, which comprises administering an effective amount of a composition of the invention comprising at least one peptide having T cell activity derived from a myelin antigen preferably in non-immunogenic form, in a therapeutic regimen which also includes the administration of IFN-β. Preferred compositions include those compositions of the invention discussed earlier comprising MBP peptides as well as MBP peptides combined with MOG peptides, in conjunction with simultaneously or sequentially administered IFN-β.

Administration of a composition of the invention comprising at least one peptide having T cell activity of a myelin antigen in a therapeutic regimen which includes administration of IFN-β can be carried out using known procedures at dosages and for periods of time to effectively reduce, eliminate or prevent the symptoms associates with multiple sclerosis. Effective amounts of either peptide or IFN-β when administered together in a therapeutic regimen vary according to factors discussed above. The active compounds (e.g. the MBP peptide or composition thereof and IFN-β) may be administered in a convenient manner such as by injection (subcutaneous), intravenous etc.), oral administration, inhalation, transdermal application or rectal administration as discussed above.

For example, preferably about 1 µg-3mg and more preferably about 20-500 µg of peptide derived from a myelin antigen per dosage unit may be administered by injection. Preferably, a dosage unit of 100-10,000 units of IFN-β may be administered by injection. The dosage regimen of these two compounds may be adjusted to provide the optimum therapeutic response. For example, IFN-β and a composition of the invention may be administered simultaneously or may preferably

be administered at least six hours apart, preferably at least 12 hours apart, or more preferably at least 24 hours apart. The therapeutic regimen of administering both antigenic peptide and IFN-β may continue over a period of days or weeks and may be reduced or extended as indicated by the exigencies of the therapeutic situation as discussed above.

The present invention also provides a novel composition comprising a physical mixture of a peptide having T cell activity derived from a myelin antigen such as MBP or MOG and IFN-β in a pharmaceutically acceptable carrier or diluent. This composition may be used as part of a therapeutic regimen for treating or preventing multiple sclerosis in an individual.

Other autoimmune diseases such as Type I diabetes and rheumatoid arthritis are generally accepted as being the result of an antigen specific T cell mediated response against an autoantigen. Various approaches to treating T cell mediated autoimmune diseases include administration of whole autoantigens or T cell epitope-containing peptides derived therefrom to the patient for the purpose of down regulating the T cell mediated response responsible for the adverse symptoms of the autoimmune disease

In addition to myelin autoantigens which play a role in MS, a number of antigens (i.e. autoantigens) have been found to cause disease symptoms in other autoimmune diseases such as diabetes, Graves disease, myasthenia gravis, Good Pasture's syndrome, psoriasis, thyroiditis, and rheumatoid arthritis (e.g. autoantigens such as insulin; rh factor; acetylcholine receptors; thyroid cell receptors; basement membrane proteins; thyroid proteins; ICA-69 (PM-1); glutamic acid decarboxylase (64K or 65 K); proteolipid, Collagen (Type II), Heat Shock Protein and carboxypeptidase H). It is believed that compositions and methods similar to those described herein may be used to treat autoimmune diseases such as rheumatoid arthritis, diabetes, myasthenia gravis, Grave's disease, Good Pasture's syndrome, psoriasis, and thyroiditis, wherein the antigen responsible for the disease is a protein autoantigen.

Peptides comprising defined sequences of amino acid residues having T cell activity and preferably comprising at least one T cell epitope and/or which induce T cell nonresponsiveness or reduced T cell responsiveness, have been identified and isolated for many of the above named autoantigens. For example, peptides which are believed to be able to down regulate the antigen specific response to soluble Type II collagen, a protein antigen believed to be an autoantigen in rheumatoid arthritis, have been identified in WO 94/07520. WO 92/06704 describes methods for identifying

peptides of insulin which are believed to comprise T cell epitopes and which may be useful in compositions and the methods analogous to the those of the of the present invention to treat Type I diabetes. In addition, suitable T cell epitope-containing peptides may be identified for an antigen or autoantigen presumed to be responsible for any of the above-named diseases by any of the procedures described above and in the examples for identifying T cell epitope containing peptides of a protein autoantigen. Once such T-cell epitope containing peptides have been identified for the targeted autoantigen, such peptides may be used in compositions and methods analogous to those described herein for treating multiple sclerosis.

This invention is illustrated by the following non-limiting examples.

EXAMPLE 1 Human Population Study of Multiple Sclerosis Immune Response to Myelin Basic Protein and Peptides and Selection of MBP Peptides Suitable for Therapeutic Use.

Peptide Synthesis:

Peptides were synthesized using standard Fmoc/tBoc chemistry and purified by Reverse Phase HPLC. Figure 3 shows the MBP peptides used in these studies. The peptide names or amino acid residues are consistent throughout.

Protocol: Analysis of Human PBL for reactivity with MBP and MBP peptides:

PBLs were purified from fresh peripheral blood specimens (approximately 75 cc) from 222 patients with definite MS using a Ficoll density gradient. Microtiter cultures were initiated with 2 x 10^5 PBL per well and 10 ug/ml purified human spinal cord MBP in RPMI 1640 culture medium supplemented with 5% human AB serum, penicillin-streptomycin, and L-glutamine. Cultures were supplemented with IL2 (20 units/ml) and with IL4 (5 units/ml) beginning at day 6-7. After 11-13 days, the microtiter cultures were washed, resuspended in fresh media, and split into 12 fresh microtiter wells. Autologous frozen PBLs were added as antigen presenting cells at 5 x 10^4 PBL per well.

Screening antigens were added in duplicate to the 12 replicate wells from each microtiter culture. Media was always used as a negative control, and purified human recombinant MBP at 10 ug/ml was used a a positive control. Each patient was also tested for reactivity with a maximum of 4 MBP peptides, each at a concentration of 10

uM. After 48 hours, the assays were pulsed with 0.75 uCi of ^{3}H-thymidine, and harvested after a 6-16 hour pulse.

Cultures were scored as positive for each peptide according to the following criteria: stimulation index greater than 3.0, change in cpm greater than or equal to 500, and standard error of the mean less than the change in cpm. In addition, for the purposes of the analysis, cultures were scored as "peptide-positive" only if they responded to both MBP and to the peptide, and if they did not respond to more than one non-overlapping peptide. Reactivity with each of the peptides was tested using a minimum of 19 and a maximum of 43 patients.

Results:

An average of 6% of the microtiter cultures scored positive for MBP reactivity for each of the patients (range 0-37 MBP positive cultures per patient). At least one microtiter culture scored positive for MBP reactivity in 77% of the patients, and these individuals were considered to be "MBP responders". MBP responder status did not correlate with gender, category of MS (RR vs. CP), HLA-DR type, age, or whether or not the patient was taking Betaseron.

MBP peptides tested included a panel of 16 20-mers (Fig. 3) overlapping by 10 amino acids and covering the entire human MBP sequence (18.5 kD isoform). Two additional longer peptides were also tested (MBP sequence (MBP 83-105 and MBP 141-165, Fig. 3). MBP peptide reactivity was then calculated based on the proportion of MBP positive microtiter cultures which also scored positive for one of the MBP peptides. Four of the peptides each accounted for at least 10% of the total MBP reactivity (MBP 11-30, MBP 81-100, MBP 83-105, and MBP 141-165). In addition, reactivity to each of these peptides was detected in at least one third of the individual MBP-responder MS patients in which they were tested. Moreover, 77% of MBP-responder patients demonstrate reativity to either MBP 83-105 or MBP 141-165, or both peptides.

MBP 141-165 was the most reactive of the four peptides, accounting for 21% of the total MBP response, and detectable in 64% of the MBP-responder patients tested. MBP 141-165 appears to include T cell epitopes from both MBP 141-160 and MBP 151-170, and surprisingly showed dramatically more reactivity than the two 20-mer peptides. MBP 81-100 and MBP 83-105 are very similar in sequence and likely to include similar if not identical T cell epitopes. They correspond to a region previously thought to be associated with the HLA-DR2 haplotype. Our results indicate that both DR2 and non-DR2 MS patients have good reactivity to these

peptides. MBP 11-30 is an area of peptide reactivity that was previously not thought to be recognized frequently in MS patients.

Each of the other MBP peptides accounted for less than 5% of the total MBP reactivity, and was detected in 20% or fewer of the MBP-responder patients tested (see, Figs 4a and 4b). The most reactivity among this group of peptides was found with MBP 111-130, which accounted for 4.5% of the MBP reactivity and was found in 19% of the MBP responders tested.

Selection of preferred MBP peptides

Preferred MBP peptides have been selected based on two criteria:

1. Number of MS patients responding to the candidate peptides (at least 75% of patients tested who respond to the protein antigen recognized at least one of the peptides).
2. Magnitude of T cell response to the candidate peptide in MS patients (response to the candidate peptides equals 40% of the total response to the antigen).

MBP 83-105 (MBP-2) and MBP 141-165 (MBP-4) meet the first criteria (77% of the MBP responders recognize one or both of them). See, Table 1 below. Together these two peptides account for 32% of the MBP reactivity (see, Table 2, below).

Table 1

Responsiveness to MBP-2 and MBP-4: Analysis by individuals

% of individuals responding to	MBP-2	MBP-4	MBP-2 and/or MBP-4
Among MBP responders	11/31 [35.5%]	20/31 [64.5%]	24/31 [77.4%]

Table 2

Responsiveness to MBP-2 and MBP-4: Analysis by T cell lines

% of MBP positive lines	MBP positive and MBP-2	MBP positive and 141-165	MBP positive and MBP-2 or MBP-4
Among MBP positive lines	20/184 [10.95%]	39/184 [21.1%]	59/184 [32.1%]

Thus, adding MBP 11-30 (MBP-1) meets the second criteria, since the three peptides together account for 42% of the MBP reactivity. MBP 111-130 (MBP-3) would also be suitable as a preferred peptide for therapeutic use particularly in combination with MBP-1, MBP-2, and MBP-4. MBP 81-100 appears equivalent to MBP 83-105 and may also be used in conjunction with the other peptides selected as preferred.

EXAMPLE 2: Administration of Peptides to Mammals for Treatment of EAE as a Model for Multiple Sclerosis

Synthesis of Peptides

Peptides were prepared by automated peptide synthesis (ABI 430A, Applied Biosciences, Foster City, CA) using standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by high pressure liquid chromatography, and amino acid

composition was confirmed. Peptide sequences selected were MBP Ac1-11 ASQKRPSQRHG; MBP Ac1-11[4A] ASQARPSQRHG; MBP Ac1-11[4Y] ASQYRPSQRHG; MBP 31-47 RHRDTGILDSIGRFFSG; Ova 323-339 ISQAVHAAHAEINEAGR; and Ova 323-337 ISQAVHAAHAEINEA.

MBP purification

MBP was prepared from guinea pig spinal cords (Keystone Biologicals, Cleveland, OH) using a modification of the method of Smith, M.E., *J. Neurochem.*, 1969, **16**:83. Briefly, MBP was extracted from isolated myelin membranes using chloroform and methanol, precipitated with potassium citrate, acid extracted, and lyophilized. SDS-PAGE analysis of this material showed a major band at the expected molecular weight of 18.5 Kd.

EAE induction, scoring, and peptide therapy

EAE was induced in (PLJ x SJL) F1 mice obtained from the Jackson Laboratory (Bar Harbor, ME). When the mice reached the age of 8-14 weeks, EAE was induced by immunization with 50-100 μg of MBP emulsified in complete Freund's adjuvant (Gibco Laboratories, Grand Island, NY), supplemented with an additional 400 μg per mouse *M. tuberculosis* H37Ra (Difco Laboratories, Detroit, MI). 200 ng pertussis toxin (JRH Biosciences, Lenexa, KS) was administered intravenously at the time of immunization and 48 hours later. Mice were scored based on clinical signs according to the following scale: 1, tail paralysis; 2, partial hind limb paralysis; 3, complete hind limb paralysis; 4, forelimb paralysis; 5, moribund or dead. Grade 5 mice were euthanized. After death, mice were excluded from subsequent calculations of mean clinical score. Peptides were administered intravenously as described in the individual examples below.

For the lymph node proliferation and IL2 production assays, inguinal and para-aortic lymph node lymphocyte suspensions were prepared from mice immunized as described above for EAE induction (pertussis toxin was not included for the proliferation experiments). Lymphocytes were cultured in round-bottom microtiter plates at 5×10^5 per well with MBP peptides and RPMI 1640 supplemented with 0.5% fresh normal mouse serum, Penicillin-Streptomycin, L-glutamine, and 5×10^{-5} M 2-mercaptoethanol. Proliferation assay cultures were pulsed for 16 hours with ^{3}H-thymidine following a 72-hour incubation. For IL2 assays, culture supernatants were harvested at 24 hours and examined for their ability to support the growth of the IL2-dependent cell line HT2. In order to distinguish IL2 production from IL4 production,

HT2 bioassays were performed in the presence of the monoclonal antibody 11.B.11 10% culture supernatant (O'Hara, J., et al., *Nature*,1985, **315**:333), which inhibits IL4-induced proliferation of the HT2 cells.

Results are shown in Figure 9 which indicates that Ac1-11[4Y] reverses EAE when administered after the onset of paralysis. EAE was induced with MBP in groups of 14 mice. Therapy was initiated in each mouse individually, after it had developed clinical signs of EAE. Data are plotted relative to initiation of therapy for each individual (day 1=onset of clinical signs for each individual mouse). Mice were treated intravenously on days 1, 4, 11, and 18 of disease with PBS (open circle), 250 nmol Ac1-11[4Y] (closed squares), or 250 nmol Ova 323-337 (closed circles). An additional control group received no treatment (open squares). Small crosses indicate individual mice which died or were sacrificed for grade 5 EAE. MMS for the untreated control group was 4.6, and mortality was 64%. MMS for the PBS treated group was 4.4, and mortality was 35%. MMS for the Ova 323-337 treated group was 4.2, and mortality was 21%. MMS for the Ac1-11[4Y] treated group was 3.2, and mortality was 7%.

Figure 10 shows that Ac1-11[4Y] prevents EAE relapses when administered during the remission phase of disease. EAE was induced with MBP. Therapy was initiated in each mouse individually, on the second day of remission after an initial episode of EAE. Data are plotted relative to initiation of therapy for each individual (day 1 = second day of remission). Groups of 6-7 surviving mice were treated intravenously on days 1, 4, 7, and 18 after the beginning of remission with either PBS (open circles) or 25 nmol Ac1-11[4Y] (closed squares). Small crosses indicate individual mice which died or were sacrificed for grade 5 EAE. Initial episodes of disease prior to remission lasted from 3 to 18 days (mean 9 days for both groups). MMS during the initial episode was similar for the two groups of mice (3.0 for the PBS treated group, and 3.9 for the Ac1-11[4Y] treated group). MMS after treatment for the PBS treated group was 4.1, and mortality was 33%. MMS after treatment for the peptide treated group was 0.3, and mortality was 0%.

Figure 11 shows that intravenous administration of Ac1-11 or Ac1-11[4Y] induces T cell non-responsiveness.

a. Groups of 3-4 mice were pretreated intravenously with either PBS (closed circles) or 250 nmol Ac1-11 (closed triangles) ten and five days prior to immunization with 150 µg MBP. Lymph node proliferation assays with MBP Ac1-11 were performed at day 9. Controls were as

follows: PBS pretreatment PPD 108277/medium 1855, and Ac1-11 pretreatment PPD 88499/medium 1335.

b. Groups of 4 mice were pretreated intravenously with either PBS or 250 nmol Ac1-11[4Y] ten and five days prior to immunization with 150μg MBP. Lymph node IL2 production assays with 150 uM Ac1-11 or 17 uM Ac1-11[4Y] were performed at day 10.

EXAMPLE 3

Administration of peptide in advanced stage

Relapsing EAE develops in (PLJxSJL)F1 mice following immunization with MBP (Fritz, R.B., et al., *J. Immunol.*, 1983, **130**:1024), making this EAE model ideal for investigating therapeutic intervention. Although MBP Ac1-11 is the immunodominant encephalitogen in (PLJ x SJL)F1 mice, the subdominant epitope MBP 31-47 is also encephalitogenic in this strain. In order to determine whether one or both of these encephalitogenic peptides is required for treatment of MBP-induced EAE, the steps of Example 2 were followed except groups of mice were treated in an advanced stage of disease with intravenous injections of MBP Ac1-11 and MBP 31-47 together, or with Ac1-11 alone. Groups of control mice were treated with intravenous injections of PBS, or with the control peptide Ova 323-339, an $A\alpha^{u}A\beta^{u}$ binding peptide which is unrelated to MBP and which is non-immunogenic in (PLJxSJL)F1 mice.

Figure 5 shows that repeated intravenous injections of MBP Ac1-11, either alone (Figure 5a) or in combination with MBP 31-47 (Figure 5b), reduce the incidence, severity, and mortality of EAE. Treatment with the two peptides appeared to be somewhat more effective than treatment with MBP Ac1-11 alone, consistent with the observations of others mentioned above. Mice were treated with six intravenous injections of peptide throughout the acute and first relapse phases. Mice then were followed until day 125, during the chronic, remitting phase, with no evidence of late disease in the MBP peptide-treated groups (data not shown). The control Ova 323-339 peptide had no effect on disease when administered by the intravenous route (Figure 5c). In a separate experiment, intravenous administration of MBP 31-47 alone was not effective in treating EAE (data not shown). Figs. 5(a), (b) and (c) show the efficacy of intravenous treatment of mice with EAE induced with MBP. EAE was induced with MBP as set out above in groups of 11-16 mice. Mice were treated with intravenous injection of 250 nmol of each peptide beginning on days 9, 12, 15, 21, 29 and 37. Small crosses as used on all figures herein indicate

individual mice which died or were sacrificed for grade 5 EAE. The control PBS-treated group (closed circles) The results indicate that the immunodominant encephalitogenic peptide of MBP is both necessary and sufficient to treat EAE induced with MBP in (PLJ x SJL)F1 mice as indicated by a reduction in severity and mortality of EAE in treated mice.

EXAMPLE 4

Multiple I.V. injections of Ac1-11

Following the preparation steps in Example 2, multiple injections of Ac1-11 were prepared. One injection of peptide at day 9 delayed disease for two days, and reduced mortality from 100% to 63%, but all of the treated mice ultimately developed severe EAE (data not shown). Three injections of peptide during the acute phase of EAE initially treated disease effectively, however, during the relapse phase the treated group of mice developed late-onset disease (data not shown). Taken together, the results indicate that multiple intravenous injections of MBP peptides during the acute and first relapse phases of EAE are required for treating MBP-induced disease in (PLJ x SJL)F1 mice. Thus, multiple injections of Ac1-11 are required for a prolonged reduction in disease severity. Figure 6 shows that Ac1-11[4Y] treats EAE at a lower dose than Ac1-11, and has a longer-lasting effect on disease. EAE was induced with MBP in groups of 8-10 mice. Figure 6a shows mice treated I.V. on days 12 and 15 with PBS (Phosphate Buffered Saline) (open circles), 250 nmol of Ac1-11(closed squares), or 250 nmol Ac1-11[4Y] (open triangles). MMS for PBS treated mice was 4.4, and mortality was 60%. MMS for Ac1-11 treated mice was 3.9, and mortality was 37.5%. MMS for Ac1-11[4Y] treated mice was 2.1, and mortality was 0%. Figure 6b shows mice treated I.V. on days 12, 115, 18, 21, 24, and 27 with either PBS (open circles) or 2.5nmol Ac1-11 (closed circles). MMS for PBS treated mice was 2.9, and mortality was 20%. MMS for peptide treated mice was 4.3, and mortality was 50%.

EXAMPLE 5

Treatment with a MBP peptide analog which forms stable complexes with class II MHC *in vivo*

Since peptide-MHC complexes are the functional therapeutic units which treat EAE, Applicants theorized that Ac1-11[4Y] would be more potent in treating active MBP-induced EAE than Ac1-11. To test this hypothesis, the steps of Example 2 were carried out except the injection schedule and dosage was changed. Figure 6a shows

that only two injections of Ac1-11[4Y] early in the disease course produce a longer lasting effect than two injections of Ac1-11. Moreover, Figure 6b shows that repeated intravenous injections of Ac1-11[4Y] are highly effective at treating EAE at a dose of 2.5 nmol (Figure 6c). Thereafter, histological tests were performed. Histological sections from EAE brains and spinal cords were prepared and stained with hematoxylin and eosin (CVD, Inc., West Sacramento, CA). Sections were scored for inflammatory infiltrates on a scale of 1-4 by a blinded observer. Efficacy of Ac1-11[4Y] was confirmed by histological examination of brain and spinal cord sections from peptide treated and PBS treated mice, which show markedly reduced number and severity of inflammatory central nervous system infiltrates in the peptide treated mice (Figure 7). Figure 7 demonstrates that inflammatory infiltrates are reduced in mice treated with AC1-11[4Y]The improved potency of Ac1-11[4Y] was evident.

The improved potency of Ac1-11[4Y] was evident in the Example above. Applicants theorized that stable peptide-MHC complexes are formed *in vivo* between $A\alpha^{u}A\beta^{u}$ and Ac1-11[4Y] which has been injected intravenously thereby causing the observed improved potency. These *in vivo*-formed complexes are detectable using the 1934 hybridoma, which was derived from an encephalitogenic MBP-specific T cell clone. The steps in the above Example 2 were followed except injection times and amounts were adjusted as described below. The 1934 T cell hybridoma is specific for MBP Ac1-11 following the procedure outlined in Wraith, D.C., et al., *Cell* 1989, 59:247. Briefly, hybridoma cells were incubated at 5×10^4 per well in flat-bottom microtiter plates with MBP peptides or peptide analogs. 5×10^5 (PLJ x SJL)F1 spleen cells, irradiated at 3000 rads, were added as antigen presenting cells. Medium was the same as for the lymph node proliferation assays, except the serum supplement was 10% fetal calf serum rather than normal mouse serum. Supernatants were harvested at 24 hours and examined for their ability to support the growth of HT2 cells. Thereafter, spleens were removed from injected mice at various times after injection of 250 nmoles Ac1-11[4Y], and the splenocytes used an antigen presenting cells for the Ac1-11-specific hybridoma 1934. No additional peptide was added to the cultures. Figure 8 shows that spleen cells which have been "pulsed" *in vivo* with Ac1-11[4Y] are highly effective at activating the 1934 hybridoma. Capacity to activate the hybridoma is maximal two hours after injection, then begins to decline by four to six hours after injection. Activation is minimal by ten hours after injection of Ac1-11[4Y], and is not present by twenty hours (data not shown). As can be seen from Figure 8, experiments with Ac1-11 show no activation of the hybridoma by spleen cells from injected mice, even at one hour following injection (data not shown). As

shown in more detail in Figure 8, peptide-MHC complexes formed in vivo are detectable in mice injected with Ac1-11[4Y]. Groups of 2 mice were injected intravenously with 250 nmol Ac1-11 4Y] at various time points prior to removal of spleens (1-10 hours). Splenocytes from the injected mice were examined for their capacity to activate the 1934 hybridoma cells to produce IL2. Results are expressed in terms of HT2 proliferation. The results support the hypothesis that formation of stable peptide-MHC complexes *in vivo* contributes to the potency of a therapeutic MBP peptide analog.

EXAMPLE 6

Synthesis of mouse MBP peptide Ac 1-11

Mouse MBP peptide Ac 1-11 was synthesized using standard Fmoc/tBoc synthesis and purified by HPLC. The amino acid sequence for peptide Ac 1-11 is as follows:

Induction of EAE

EAE was induced in 6 to 8 week old female (SJL x PL)F_1 mice (Jackson Labs, Bar Harbor, ME) by immunizing mice with 100 µg purified guinea pig MBP in CFA (GIBCO Lab., Grand Island, NY) containing 400 µg H37RA strain M. tuberculosis (DIFCO Lab., Detroit, MI) subcutaneously at the base of the tail. 200 ng Pertussis Toxin (JHL BIOSCIENCE, Lenexa, Kansas) was given twice intravenously (i.v.) on the day of immunization and also 48 hours later. Mice were monitored daily for disease symptoms and were scored for disease severity on the following scale 0=no clinical signs of EAE, 1=limp, unresponsive tail, 2=partial hindlimb paralysis, 3=complete hindlimb paralysis, 4=partial to complete forelimb paralysis and 5=moribund. Data are expressed as the mean of the disease severity score on each day including all the animals in the group. Mice were followed for 26 days. Once a mouse died of EAE, a score of 5 was included in calculations for all subsequent days.

Effect of IFN-β on EAE

In a titration experiment for the purposes of determining the effects of various dosages of IFN-β on EAE (Fig. 13). One group of mice was treated intraperitoneally with PBS on days 9, 13, and 16 (control) after EAE induction, one group of mice were treated with 10,000 units of IFN-β on days 9, 13, and 16 (open circle) and one group of mice were treated with 2,000 units of IFN-β on days 9, 13, and 16 (closed circle). As shown in Fig. 13, the symptoms of EAE are similar at each time point for both

dosages of IFN-β, thereby indicating that the lower dosage would be suitable for experiments with IFN-β and is a preferred dosage as the chances of toxicity due to a higher dose of IFN-β are less likely. 2000 units of IFN-β were then used in the remaining experiments shown in Figs 12a-12c as this dosage showed improvement in clinical score.

As shown in Fig 12a, a control group of mice was treated with PBS and another group of mice were treated with 2000 units of IFN-β i.p. on days 9, 12, 16, and 20. As shown in Fig. 12a, the group treated with IFN-β only had slightly less severe symptoms during the time course as those of the control group.

Effect of mouse MBP peptide Ac 1-11 on EAE

The effect of mouse MBP peptide Ac 1-11 was determined and the results are shown in Fig. 12b. One group of mice was treated i.p. with PBS on days 10, 13, 17, and 21 (control) after EAE induction, and one group of mice was treated i.v. with 250 nmol of peptide Ac 1-11 on days 10, 13, 17, and 21. The mice were monitored as described above. As shown in Fig 12b, the mice treated with Ac 1-11 had less severe symptoms than those of the control group.

Effect of treatment with a combination of peptide Ac 1-11 and IFN-β on EAE

The effects of treatment with a combination of peptide Ac 1-11 and IFN-β are shown in Fig. 12c. One group of mice was treated i.p. with PBS (control) after EAE induction, and one group of mice was treated i.v. with 250 nmol of peptide Ac 1-11 on days 10, 13, 17 and 21 (open arrows) and treated i.p. with 2000 units of IFN-β on days 9, 12, 16, and 20. As shown in Fig. 12c, the group of mice treated with a combination of peptide and IFN-β showed a marked decrease in the severity of symptoms as compared with the control group as well as compared to treatment with either IFN-β alone or peptide alone as shown in Figs. 12a and 12b indicating a synergistic effect of the combination. Therefore, a treatment regimen which includes a combination of peptide and IFN-β provides an enhanced effect on diminishing the severity of the symptoms of EAE.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

EXAMPLE 7: Administration of Peptides to Mammals for Treatment of Spinal Cord Homogenate (SCH) Induced EAE as a Model for Multiple Sclerosis

Synthesis of Peptides

Peptides were prepared by automated peptide synthesis (ABI 430A, Applied Biosciences, Foster City, CA) using standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by high pressure liquid chromatography, and amino acid composition was confirmed. Peptide sequences selected were MBP Ac1-11 ASQKRPSQRHG; MBP Ac1-11[4A] ASQARPSQRHG; MBP Ac1-11[4Y] ASQYRPSQRHG; MBP 31-47 RHRDTGILDSIGRFFSG; Ova 323-339 ISQAVHAAHAEINEAGR; and Ova 323-337 ISQAVHAAHAEINEA.

MBP purification

SCH was prepared from guinea pig spinal cords (Keystone Biologicals, Cleveland, OH) using a modification of the method of Smith, M.E., *J. Neurochem.*, 1969, **16**:83. The SCH protein mixture was lyophilized and used according to dry weight.

EAE induction, scoring, and peptide therapy

EAE was induced in (PLJ x SJL) F1 mice obtained from the Jackson Laboratory (Bar Harbor, ME). When the mice reached the age of 8-14 weeks, EAE was induced by immunization with 500-1000 μg of SCH emulsified in complete Freund's adjuvant (Gibco Laboratories, Grand Island, NY), supplemented with an additional 400 μg per mouse *M. tuberculosis* H37Ra (Difco Laboratories, Detroit, MI). 200 ng pertussis toxin (JRH Biosciences, Lenexa, KS) was administered intravenously at the time of immunization and 48 hours later. Mice were scored based on clinical signs according to the following scale: 1, tail paralysis; 2, partial hind limb paralysis; 3, complete hind limb paralysis; 4, forelimb paralysis; 5, moribund or dead. Grade 5 mice were euthanized. After death, mice were excluded from subsequent calculations of mean clinical score. Peptides were administered intravenously as described in the individual examples below.

Results are shown in Figure 16 which indicates that Ac1-11[4Y] reverses EAE when administered after the onset of paralysis. EAE was induced with SCH in groups of 14 mice. Therapy was initiated in each mouse individually, after it had

developed clinical signs of EAE. Data are plotted relative to initiation of therapy for each individual (day 1=onset of clinical signs for each individual mouse). Mice were treated on days 1, 3, 6, and 9 of disease with PBS (closed square), 250 nmol Ac1-11[4Y] i.v. (open triangles), or 250 nmol AcI[4Y] (open circles). A small asterisk indicates individual mice which died or were sacrificed for grade 5 EAE.

Figure 17 shows that Ac1-11[4Y] prevents EAE onset when administeredbefore symptoms of the disease are exhibited. EAE was induced with SCH. Therapy was initiated in each mouse individually, on the eighth day after disease induction with gpSCH. Data are plotted relative to initiation of therapy for each individual. Groups of surviving mice were treated on days 8, 10, 13, and 17. 25 nmol Ac1-11[4Y] i.v.(open triangles) or 25 nmol Ac1-11p4Y] s.c. (closed circles). One group received no treatment (open squares).

Results shown on figs. 16 and 17 show that either route of administration is effective in preventing onset of symptoms.

EXAMPLE 8

The same procedure was followed as in Example 2. however, no lymph node proliferation and IL2 production assays were performed. EAE was induced using gpMBP on day 0, thereafter Ac1-11[4Y] was administered to 3 groups of mice on day 8, 10, 13, and 17. The results are graphically represented on Fig. 18 for intravenous administration (open triangles), subcutaneous injection (closed circles) and a control (open circles). Pretreatment (e.g. treatment before display of symptoms) with Ac1-11[4Y] can prevent gpMBP induced EAE disease.

EXAMPLE 9 cDNA Encoding Human MOG Protein

In an initial attempt to obtain human DNA encoding MOG protein, a human cDNA library was subjected to the polymerase chain reaction (PCR) using 3' and 5' primers designed from the published rat MOG coding sequence of Gardinier *et al.* (*supra*). The human MOG sequence could not be obtained in this manner, putatively due to insufficient homology at the 5' and/or 3' ends of the human and rat sequences.

Therefore, four rat internal oligonucleotides were designed. Two of them were homologous to the top strand of the gene (primers 94-111 and 166-183 (SEQ ID NO: 34), base 1 starting at the ATG) and two were homologous to the bottom strand of the gene (primers 538-555 (SEQ ID NO: 35) and 685-702). The combination of primers 166-183 (SEQ ID NO: 34) and 538-555 (SEQ ID NO: 35) was successful in effecting the amplification of a fragment of the approximately 400

bp expected size from a human brain cDNA library. The sequence of these primers was:

a) 166-183: CAGAATCCGGGAAGAATGCCACGGGC (SEQ ID NO: 34); and

b) 538-555: CAGCGGCCGCACGGAGTTTTCCTCTCAG (SEQ ID NO: 35).

An EcoRI site is present in the 166-183 primer (SEQ ID NO: 34); a NotI site is present in the 538-555 primer (SEQ ID NO: 35).

The 400 bp PCR product was cloned into expression vector pVL1393 by digesting pVL1393 (Pharmingen CA) with EcoRI and NotI, digesting the amplified product with the same enzymes and ligating the resulting fragments. The insert was verified by digesting several clones derived from the ligated plasmids with EcoRI and NotI and sequencing the resulting 400 bp human MOG fragment. The resulting insert putatively lacks 184 bp of 5' sequence and 201 bp of 3' sequence, based on the 738 bp rat open reading frame.

Two primers were designed from the 400 bp insert from positions 346-363 top and bottom strands as follows:

5'-CAGAATTCTCAGGTTCTCAGATGAAGGA-3' (SEQ ID NO: 36); and

5'-AAGCGGCCGCTATCCTTCATCTGAGAACCT-3' (SEQ ID NO: 37).

wherein an EcoRI site is present in the first strand and a NotI site in the second. Underlined regions correspond to the MOG sequence.

The human MOG 346-363 top and bottom primers (SEQ ID NOS: 36 and 37) were used in combination with the above-mentioned 5' and 3' rat primers, respectively, to amplify the 5' and 3' missing ends of the gene from the same human brain cDNA library as previously used. A PCR product corresponding the 3' end of the gene was obtained, but the corresponding 5' end did not result.

The 3' fragment obtained had the expected 400 bp size and this fragment was cloned in pVL1393 and sequenced.

To obtain the 5' portion of the gene, a human brain medulla λgt10 library obtained from Clontech which had been previously amplified and had a titer of $8x10^{10}$ pfu/ml was screened following the protocol described by the manufacturer. The library was plated onto 12 large plates at 30,000 plaques/plate and the plaques were lifted onto nitrocellulose filters (2 replica filters/plate). Twelve filters lifted from the 12 different plates were then hybridized to a ^{32}P labelled probe corresponding

to the human MOG internal 400 bp fragment initially cloned (positions 184-534). Twenty-two strong positives were obtained. A plug was picked for each positive from the original plates and incubated overnight with λ dilution buffer to elute the phage from the agar. The tube was then centrifuged and the supernatant transferred.

The DNA was amplified from each individual pool using either a λ gt10 forward primer with an SstII site:
5'-CTTTTGAGCAAGTTCAGCCTGGTTAAG-3' (SEQ ID NO: 38) or a λgt10 reverse primer with an XhoI site:
5'-ACCTCGAGGAGGTGGCTTATGAGTATTTCTTCCAGGGTA-3' (SEQ ID NO: 39) as well as a human MOG internal primer top or bottom strand:
5'-GGTGCGGGAAAGGTGACTCTCAGGATCCGGAAT-3' (SEQ ID NO: 40) or
5'-ATTCCGGATCCTGAGAGTCACCTTTCCCGCACC-3' (SEQ ID NO: 41).

The last two primers (SEQ ID NOS: 40 and 41) include a BamHI site (underlined in the sequences) naturally present in the human MOG sequence.

The primers were used in four different combinations: 1) forward top/internal MOG bottom; 2) reverse bottom/internal MOG bottom; 3) internal MOG top/reverse bottom; and 4) internal MOG top/forward top.

The first two combinations provided the 5' end of the gene (up to the BamHI site) and the last two, the 3' end of the gene. Both 5' and 3' portions include untranslated regions. Which of the two members of each combination actually resulted in the desired fragment depends on the orientation of the cDNAs cloned into λgt10.

The size of the fragments obtained varied from one pool to another. Five of the largest 5' fragments or 3' fragments were subcloned into the SstII and BamHI or BamHI and XhoI sites of the SK polylinker. Three clones from each pool were then sequenced to rule out the presence of PCR errors. This provided the complete sequence of the gene coding region as well as 174 bp of the 5' untranslated sequence.

The complete DNA sequence recovered (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) are shown in Figure 1.

The human MOG gene encodes a preprotein of 248 amino acids which has 87% homology with the 246 amino acids in the rat protein. The mature protein contains 218 amino acids, numbered 1-218 in Figure 1 (SEQ ID NO: 2). The mature protein begins at the glycine shown at position 1 and is derived from the 248 amino

acid preprotein by cleavage from the presequence extending from the MET start codon to the alanine residue immediately preceding the glycine shown in position 1.

EXAMPLE 9A **Expression of truncated human MOG in SF-9 Insect cells and *E. coli***

SF-9 Expression

The PVL1393 transfer vector containing the truncated human MOG cDNA encoding amino acids 1-121 of human MOG (the first 121 amino acids of SEQ ID NO: 2) was cotransfected into SF-9 cells along with Baculogold linearized Baculovirus DNA (Pharmingen, San Diego, CA). The culture supernatant containing recombinant viruses was harvested after 4 days. The recombinant virus was plaque purified and subjected to 3 rounds of amplification to obtain a high titer viral stock. AF-9 cells were then infected with the viral stock at a MOI of 2.0. The supernatant from infected cells was harvested 48 hours after infection and applied to a NiNTA agarose column. The recombinant MOG protein was eluted under non-denaturing conditions using 250 mM Imidazole, dialyzed against 5% propionic acid and H_2O and subsequently lypophilized. The protein concentration was estimated by BCA. The purified MOG protein was visualized on a 12.5% polyacrylamide gel stained with Coomassie blue.

EXAMPLE 10

Truncated human MOG (huMOG) was prepared as disclosed in Examples 9 and 9A. MBP (guinea pid) was preapred as disclosed. The procedures of previous Example 2 was followed except EAE was induced in three separate groups of mice by using 75 µg gpMBP and 100 µg huMOG (open triangles) and a combination of 75 µg gp MBP and 100 µg huMOG (closed squares). The graph in Fig. 19 shows the course of disease for each for this example. The disease induced with both MOG and MBP is much more severe than the disease induced by either alone. The results indicate that both MOG and MBP contribute to the disease.

EXAMPLE 11

Disease was induced as in Example 10 using huMOG +gpMBP (Day 0 = disease induction). Mice were treated before onset of symptoms with 250 nmoles of Ac1-11[4Y] and a control (PBS) on days 6, 8, 10, 13, 17, 22 and 27 (arrows indicate treatment). As the graph in Fig. 20 indicates, treatment with Ac1-11[4Y] (open

spaces) significantly reduced the mean clinical score as compared to controls (closed squares).

Claims

What is claimed is:

1. A composition for treating multiple sclerosis in a mammal comprising at least one peptide, said peptide selected from the following group of peptides: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4 and MBP-5 all as shown in Fig. 2, or modifications or analogs or peptidomimetics thereof.

2. The composition of claim 1 wherein said at least one peptide is selected from the group consisting of MBP-1.1, MBP-2.1, MBP-4 and MBP-5 all and as shown in Fig. 2.

3. The composition of claim 1 wherein said at least one peptide is MBP-4.

4. A composition for treating multiple sclerosis in a mammal comprising at least one peptide derived from MBP selected from the group consisting of : MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4 and MBP-5 all as shown in Fig 2, wherein said composition comprises at least 40% of the total T cell reactivity to MBP in a population of individuals having T cells which respond to MBP.

5. A composition for treating multiple sclerosis in a mammal comprising at least two peptides of MBP selected from the group consisting of: MBP-1, MBP-1.1, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4 and MBP-5 all as shown in Fig 2.

6. A composition of claim 5 wherein said at least two peptides are selected from the group consisting of: MBP-1.1, MBP-2.1, MBP-4 and MBP-5 all as shown in Fig. 2.

7. A composition of claim 5 wherein one of said at least two peptides comprises MBP-4.

8. A composition of claim 5 further comprising at least one peptide selected from the group consisting of amino acid sequences: 13-25, 31-50, 61-80, 82-92, 82-96, 82-

97, 82-98, 82-100, 82-100 [100 P>Y], 83-100, 83-101, 84-97, 84-100, 84-100 , 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, all as shown in Fig. 14.

9. A composition of claim 5 further comprising at least one peptide selected from the group consisting of amino acid sequences: 13-25, 87-99, 87-99 [91K>A], 82-100, 82-100 [100P>Y] all as shown in Fig. 14.

10. A composition for treating multiple sclerosis in a mammal comprising at least two peptides derived from MBP wherein at least one peptide is MBP-4 and at least one peptide is selected from the group consisting of: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-5, 13-25, 31-50, 61-80, 82-92, 82-96, 82-97, 82-98, 82-100, 82-100 [100 P>Y], 83-100, 83-101, 84-97, 84-100, 84-100 , 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, all as shown in Figs. 2 and 14.

11. The composition of claim 10 wherein at least one peptide is MBP-4 and at least one peptide is selected from the group consisting of: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-5, 13-25, 87-99, 87-99 [91K>A], 82-100, 82-100 [100P>Y].

12. A composition for treating multiple sclerosis in a mammal comprising at least two peptides of MBP selected from the following group of peptides: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, and MBP-5 all as shown in Fig 2., wherein said composition comprises at least 40% of the total T cell reactivity to MBP in a population of individuals having T cells which respond to MBP.

13. A composition of claim 5 wherein said composition comprises a sufficient percentage of the total T cell reactivity to MBP such that administration of the composition to an individual with MS results in down regulation of the MS autoimmune response.

14. A composition for treating multiple sclerosis in a mammal comprising at least two T cell epitope-containing peptides of MBP, said composition selected from the group of compositions consisting of:

MBP-1, MBP-2, MBP-3, and MBP-4, and MBP-5;

MBP-1.1, MBP-2.1, MBP-3, MBP-4, and MBP-5

MBP-1.1, MBP-2, MBP-4, and MBP-5;

MBP-1, MBP-2.1, MBP-4, and MBP-5;

MBP-1, MBP-2, MBP-4, and MBP-5;

MBP-1.1, MBP-2.1, MBP-4, and MBP-5;

MBP-1.1, MBP-2.1, and MBP-4;

MBP-1, MBP-2.1, and MBP-4;

MBP-1.1, MBP-2, and MBP-4;

MBP-1.1, MBP-2.1, and MBP-5;

MBP-1.1, MBP-2.1, and MBP-3;

MBP-1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-1.1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-4, and a selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;

MBP-4, and a peptide selected from the group consisting of: MBP-1, or MBP-1.1;

MBP-1.1, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;

MBP-1.1, and MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;

MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;.

MBP-1.1, MBP-3, MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

15. A composition of claim 5 further comprising at least one peptide having T cell activity derived from human myelin oligodendrocyte protein (MOG) protein.

16. A composition of claim 15 wherein said peptide derived from MOG is selected from the group consisting of:

Human MOG 1-13	GQFRVIGPRHPIR;
Human MOG 103-115	HSYQEEAAMELKV;
Human MOG 1-121	GQFRVIGPRHPIRALVGDEV ELPCRTSPGKNATGMEVGWY RPPFSRVVHLYRNGKDQDGD QAPEYRGRTELLKDAIGEGK VTLRIRNVRFSDEGGFTCFF RDHSYQEEAAMELKVEDPFYW;
Human MOG 1-20	GQFRVIGPRHPIRALVGDEV;
Human MOG 11-30	PIRALVGDEVELPCRISPGK;
Human MOG 21-40	ELPCRISPGKNATGMEVGWY;
Human MOG 31-50	NATGMEVGWYRPPFSRVVHL;
Human MOG 141-160	TVGLVFLCLQYRLRGKLRAE;
Human MOG 151-170	YRLRGKLRAEIENLHRTFDP;
Human MOG 161-180	IENLHRTFDPHFLRVPCWKI; and
Human MOG 199-218	YNWLHRRLAGQFLEELRNPF.

17. A composition of claim 14 further comprising at least one peptide having T cell activity derived from MOG.

18 An isolated peptide derived from MBP wherein said peptide is selected from the group consisting of: MBP-1.1, MBP-1.2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3.1, MBP-4, and MBP-5 all as shown in Fig. 2, or a modification or analog thereof.

19. An isolated peptide of claim 18 wherein said peptide is MBP-4 or a modification or analog thereof.

20. An analog of a peptide of claim 18 wherein at least one amino acid residue of said peptide is substituted with alanine, glutamic acid or a methyl amino acid.

21. A peptide selected from the group consisting of MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6 all as shown in Fig. 2, wherein lysine (K) is substituted with alanine (A).

22. A peptide analog of MBP-2.1 wherein lysine (K) at position 10 is substituted with alanine (A), said peptide analog having the amino acid sequence DENPVVHFFANIVTPRTPPPSQGK.

23. A peptidomimetic of a peptide selected from the group consisting of: MBP-1. MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, and MBP-5 wherein at least one normal peptide bond is substituted with a non peptide bond, a peptide bond analog, or a reduced bond analog.

24. A peptide selected from the group consisting of: MBP-1. MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, and MBP-5, which has been modified by the addition of at least one charged amino acid residue to the amino terminus, carboxy terminus, or both of said peptide to increase the solubility of said peptide in an aqueous solution.

25. A peptide selected from the group consisting of: MBP-1. MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, and MBP-5, which has been modified by the addition of at least one amino acid residue to the amino terminus, carboxy terminus or both of said peptide to increase the T cell activity of said peptide, said at least one amino acid residue being derived from the native MBP protein sequence..

26. A method for treating multiple sclerosis in a mammal comprising administering to said mammal a composition of claim 1, in an amount sufficient to down regulate the autoimmune response in multiple sclerosis.

27. The method of claim 26 wherein said administration is selected from the following routes of administration: i.v. injection in non immunogenic form, subcutaneous injection in non immunogenic form, oral administration, inhalation administration, sublingual administration, transdermal administration, rectal administration or any combination thereof.

28. The method of claim 26 wherein said administering is subcutaneous administration in non immunogenic form.

29. A method of preventing the onset of multiple sclerosis in a mammal susceptible to multiple sclerosis comprising administering to said mammal a composition of claim 1, in an amount sufficient to prevent the onset of the symptoms of multiple sclerosis.

30. A method for treating multiple sclerosis in a mammal comprising administering to said mammal a composition of claim 5, in an amount sufficient to down regulate the symptoms of multiple sclerosis.

31. A method for treating multiple sclerosis in a mammal comprising administering to said mammal a composition of claim 10, in an amount sufficient to down regulate the symptoms of multiple sclerosis.

32. A method for treating multiple sclerosis in a mammal comprising administering to said mammal a composition of claim 14, in an amount sufficient to down regulate the symptoms of multiple sclerosis.

33. A method for treating multiple sclerosis in a mammal comprising administering to said mammal a composition of claim 15, in an amount sufficient to down regulate the symptoms of multiple sclerosis.

34. A method for treating multiple sclerosis in a mammal comprising administering simultaneously or sequentially at least two different compositions of claim 1, in an amount sufficient to down regulate the symptoms of multiple sclerosis.

35. A treatment regimen for treating multiple sclerosis in a mammal comprising administering simultaneously or sequentially, as a single treatment episode, a combination of peptides derived from MBP selected from the group of peptide combinations consisting of:
MBP-1, MBP-2, MBP-3, and MBP-4, and MBP-5;
MBP-1.1, MBP-2.1, MBP-3, MBP-4, and MBP-5
MBP-1.1, MBP-2, MBP-4, and MBP-5;
MBP-1, MBP-2.1, MBP-4, and MBP-5;
MBP-1, MBP-2, MBP-4, and MBP-5;
MBP-1.1, MBP-2.1, MBP-4, and MBP-5;
MBP-1.1, MBP-2.1, and MBP-4;
MBP-1, MBP-2.1, and MBP-4;
MBP-1.1, MBP-2, and MBP-4;
MBP-1.1, MBP-2.1, and MBP-5;
MBP-1.1, MBP-2.1, and MBP-3;

MBP-1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;
MBP-1.1, and a peptide selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;
MBP-4, and a selected from the group consisting of: MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6;
MBP-4, and a peptide selected from the group consisting of: MBP-1, or MBP-1.1;
MBP-1.1, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;
MBP-1.1, and MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.
MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;
MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.
MBP-1.1, MBP-5, MBP-4, and a peptide selected from the group consisting of: 82-100, 82-100 [100P>Y], 87-99, and 87-99 [91K>A], all as shown in Fig. 14;.
MBP-1.1, MBP-3, MBP-4, and a peptide selected from the group consisting of: MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5. or MBP-2.6, all as shown in Fig. 2.

36. A multipeptide formulation for pharmaceutical administration to individuals with MS comprising at least two peptides of MBP, each peptide being soluble and stable at a physiologically acceptable predetermined pH said peptides being selected from the group consisting of :MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4 and MBP-5.

37. A method of treating advanced stage multiple sclerosis in a mammal comprising administering to said mammal a therapeutic composition comprising at least one peptide having T cell activity derived from a myelin antigen, or an analog of said peptide, in an amount effective to down regulate symptoms of multiple sclerosis.

38. The method of claim 37 wherein said myelin antigen is selected from the group consisting of myelin basic protein (MBP), myelin oligodendrocyte protein (MOG), proteolipid protein (PLP), and myelin associated glycoprotein (MAG).

39. The method of claim 37 wherein said administration occurs during an acute phase of multiple sclerosis.

40. The method of claim 37 wherein said administration occurs during a remission of multiple sclerosis disease symptoms.

41. The method of claim 37 wherein said peptide comprises a peptide analog having an MHC binding affinity which is higher than the MHC binding affinity of the peptide from which the analog is derived.

42. A method of treating advanced stage multiple sclerosis in a mammal comprising administering to said mammal a therapeutic composition comprising at least one MBP peptide said peptide selected from the group consisting of: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, MBP-5, 13-25, 31-50, 61-80, 82-92, 82-96, 82-97, 82-98, 82-100, 82-100 [100 P>Y], 83-100, 83-101, 84-97, 84-100, 84-100 , 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, all as shown in Figs. 2 and 14, or an analog of said peptide, in an amount effective to down regulate symptoms of multiple sclerosis.

43. The method of claim 42 wherein said at least one peptide is selected from the group consisting of: MBP-1, MBP-1.1, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, MBP-5, 13-25, 87-99, 87-99[91K>A], 82-100, 82-100[100P>Y] or an analog thereof.

44. The method of claim 37 wherein said at least one peptide is derived from human MOG and is selected from the group consisting of:

Human MOG 1-13	GQFRVIGPRHPIR;
Human MOG 103-115	HSYQEEAAMELKV;
Human MOG 1-121	GQFRVIGPRHPIRALVGDEV ELPCRTSPGKNATGMEVGWY RPPFSRVVHLYRNGKDQDGD QAPEYRGRTELLKDAIGEGK VTLRIRNVRFSDEGGFTCFF RDHSYQEEAAMELKVEDPFYW;
Human MOG 1-20	GQFRVIGPRHPIRALVGDEV;
Human MOG 11-30	PIRALVGDEVELPCRISPGK;
Human MOG 21-40	ELPCRISPGKNATGMEVGWY;

Human MOG 31-50	NATGMEVGWYRPPFSRVVHL;
Human MOG 141-160	TVGLVFLCLQYRLRGKLRAE;
Human MOG 151-170	YRLRGKLRAEIENLHRTFDP;
Human MOG 161-180	IENLHRTFDPHFLRVPCWKI; and
Human MOG 199-218	YNWLHRRLAGQFLEELRNPF.

45. The method of claim 42 further comprising at least one peptide of human MOG selected from the group consisting of:

Human MOG 1-13	GQFRVIGPRHPIR;
Human MOG 103-115	HSYQEEAAMELKV;
Human MOG 1-121	GQFRVIGPRHPIRALVGDEV
	ELPCRTSPGKNATGMEVGWY
	RPPFSRVVHLYRNGKDQDGD
	QAPEYRGRTELLKDAIGEGK
	VTLRIRNVRFSDEGGFTCFF
	RDHSYQEEAAMELKVEDPFYW;
Human MOG 1-20	GQFRVIGPRHPIRALVGDEV;
Human MOG 11-30	PIRALVGDEVELPCRISPGK;
Human MOG 21-40	ELPCRISPGKNATGMEVGWY;
Human MOG 31-50	NATGMEVGWYRPPFSRVVHL;
Human MOG 141-160	TVGLVFLCLQYRLRGKLRAE;
Human MOG 151-170	YRLRGKLRAEIENLHRTFDP;
Human MOG 161-180	IENLHRTFDPHFLRVPCWKI; and
Human MOG 199-218	YNWLHRRLAGQFLEELRNPF.

46. The method of claim 37 wherein said administration comprises subcutaneous or intravenous injection of said peptide.

47. The method of claim 37 wherein said administering comprises increasing the dosage with each subsequent injection.

48. The method of claim 37 wherein said administering comprises decreasing the dosage with each subsequent injection.

49. A method for treating a mammal in an advanced stage of multiple sclerosis comprising administering at least one therapeutic composition comprising at least one isolated and purified peptide, said peptide having a defined length, a defined sequence of amino acid residues, and comprising T cell activity, said composition being capable of down regulating symptoms of multiple sclerosis in a population of mammals with advanced state multiple sclerosis, when administered in non-immunogenic form, and wherein said composition is soluble in an aqueous solution and stable at a physiologically acceptable pH.

50. The method of claim 49 wherein said administering comprises administering simultaneously or sequentially at least two of said therapeutic compositions.

51. A method for treating advanced stage multiple sclerosis in a mammal comprising administering a therapeutic composition of claim 5 in an amount effective to down regulate the symptoms of multiple sclerosis.

52. A method for treating advanced stage multiple sclerosis in a mammal comprising administering a therapeutic composition of claim 10 in an amount effective to down regulate the symptoms of multiple sclerosis.

53. A method for treating advanced stage multiple sclerosis in a mammal comprising administering a therapeutic composition of claim 14 in an amount effective to down regulate the symptoms of multiple sclerosis.

54. A method for treating advanced stage multiple sclerosis in a mammal comprising administering a therapeutic composition of claim 15 in an amount effective to down regulate the symptoms of multiple sclerosis.

55. The method of claim 37 wherein said advanced stage multiple sclerosis is relapsing-remitting MS, chronic progressive MS, primary progressive MS, or benign MS.

56. The method of claim 37 further comprising the step of administering β-interferon in conjunction with said therapeutic composition.

57. A method for treating multiple sclerosis in an individual comprising administering a therapeutically effective amount of at least one peptide of a myelin autoantigen having T cell activity in a therapeutic regimen which includes administering a therapeutically effective amount of IFN-β.

58. The method of claim 57 wherein said peptide and said IFN-β are administered simultaneously.

59. The method of claim 57 wherein said peptide and said IFN-β are administered at least 24 hours apart.

60. A composition comprising a therapeutically effective amount of at least peptide having T cell activity of human MBP and a therapeutically effective amount of IFN-β in a pharmaceutically acceptable carrier or diluent.

61. A composition of claim 60 wherein said at least one peptide is selected from the group consisting of: MBP-1, MBP-1.1, MBP-1.2, MBP-2, MBP-2.1, MBP-2.2, MBP-2.3, MBP-2.4, MBP-2.5, MBP-2.6, MBP-3, MBP-3.1, MBP-4, MBP-5, 13-25, 31-50, 61-80, 82-92, 82-96, 82-97, 82-98, 82-100, 82-100 [100 P>Y], 83-100, 83-101, 84-97, 84-100, 84-100 , 85-100, 86-105, 87-99, 87-99 [91K>A], 88-100, 88-99, 111-135, 122-140, 139-170, 141-160, 142-166, 142-168, 146-160, and 153-170, all as shown in Figs. 2 and 14

62. A method of preventing the onset of multiple sclerosis in an individual susceptible to multiple sclerosis comprising administering a therapeutically effective amount of at least one peptide having T cell activity of a myelin antigen in a treatment regimen which includes administering a therapeutically effective amount of IFN-β.

63. A method of preventing the onset of multiple sclerosis in an individual susceptible to multiple sclerosis comprising administering a therapeutically effective amount of at least one composition of claim 1 in a treatment regimen which includes administering a therapeutically effective amount of IFN-β.

64. A method of treating multiple sclerosis in a mammal comprising administering a therapeutically effective amount of at least one composition of claim 5 in a treatment regimen which includes administering a therapeutically effective amount of IFN-β.

65. A method of treating multiple sclerosis in a mammal comprising administering a therapeutically effective amount of at least one composition of claim 10 in a treatment regimen which includes administering a therapeutically effective amount of IFN-β.

66. A method of treating multiple sclerosis in a mammal comprising administering a therapeutically effective amount of at least one composition of claim 14 in a

treatment regimen which includes administering a therapeutically effective amount of IFN-β.

67. A method of treating multiple sclerosis in a mammal comprising administering a therapeutically effective amount of at least one composition of claim 15 in a treatment regimen which includes administering a therapeutically effective amount of IFN-β.

68. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an MHC Class II peptide complex capable of binding a T cell receptor and inducing anergy in a T cell bearing the receptor, the complex consisting essentially of:

an MHC Class II component comprising extracellular domains of an MHC Class II molecule sufficient to form an antigen binding pocket, said component being encoded by an allele associated with an autoimmune disease, which component is soluble under physiological conditions in the absence of detergent or lipid; and

the autoantigenic peptide of claim 1, the autoantigenic peptide being bound to the antigen binding pocket.

69. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an MHC Class II peptide complex capable of binding a T cell receptor and inducing anergy in a T cell bearing the receptor, the complex consisting essentially of:

an MHC Class II component comprising extracellular domains of an MHC Class II molecule sufficient to form an antigen binding pocket, said component being encoded by an allele associated with an autoimmune disease, which component is soluble under physiological conditions in the absence of detergent or lipid; and

the autoantigenic peptide of claim 8, the autoantigenic peptide being bound to the antigen binding pocket.

70. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and an MHC Class II peptide complex capable of binding a T cell receptor and inducing anergy in a T cell bearing the receptor, the complex consisting essentially of:

an MHC Class II component comprising extracellular domains of an MHC Class II molecule sufficient to form an antigen binding pocket, said component being

encoded by an allele associated with an autoimmune disease, which component is soluble under physiological conditions in the absence of detergent or lipid; and

the autoantigenic peptide of claim 16, the autoantigenic peptide being bound to the antigen binding pocket.

71. The composition of claim 68, wherein the autoimmune disease is multiple sclerosis.

72. The composition of claim 1 wherein said composition comprises tandem copies of said peptide.

73. The composition of claim 8 wherein said composition comprises tandem copies of said peptide.

74. The composition of claim 16 wherein said composition comprises tandem copies of said peptide.

Fig. 1

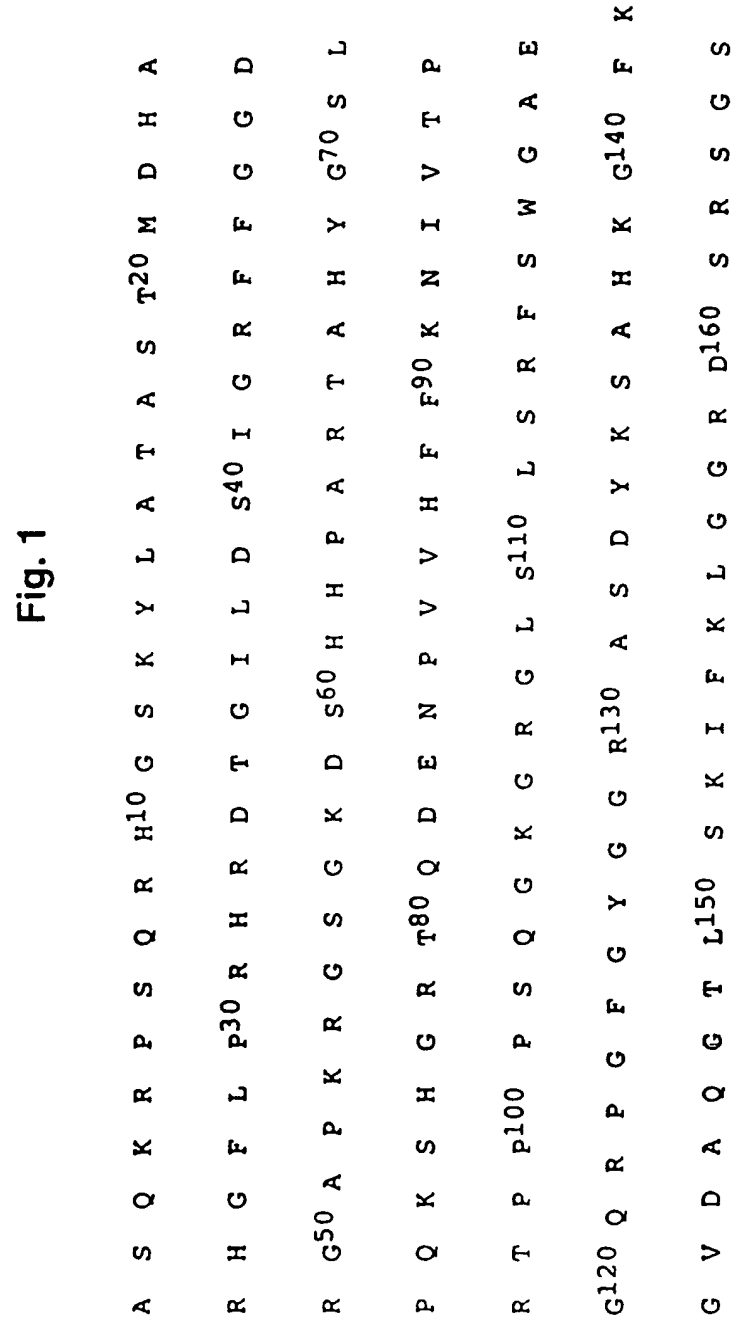

A S Q K R P S Q R H10 G S K Y L A T A S T20 M D H A
R H G F L P30 R H R D T G I L D S40 I G R F F G G D
R G50 A P K R G S G K D S60 H H P A R T A H Y G70 S L
P Q K S H G R T80 Q D E N P V V H F F90 K N I V T P
R T P P100 P S Q G K G R G L S110 L S R F S W G A E
G120 Q R P G F G Y G G G R130 A S D Y K S A H K G140 F K
G V D A Q Q G T L150 S K I F K L G G R D160 S R S G S
P M A R R170

Fig. 2

Peptide Name	Amino Acid Sequence
MBP-1 (11-30)	GSKYLATASTMDHARHGFLP
MBP-1.1 (11-29)	GSKYLATASTMDHARHGFL
MBP-1.2 (11-31)	GSKYLATASTMDHARHGFLPR
MBP-2 (83-105)	ENPVVHFFKNIVTPRTPPPSQGK
MBP-2.1 (82-105)	DENPVVHFFKNIVTPRTPPPSQGK
MBP-2.2 (82-104)	DENPVVHFFKNIVTPRTPPPSQG
MBP-2.3 (80-98)	TQDENPVVHFFKNIVTPRT
MBP-2.4 (82-102)	DENPVVHFFKNIVTPRTPPPS
MBP-2.5 (80-104)	TQDENPVVHFFKNIVTPRTPPPSQG
MBP-2.6 (80-102)	TQDENPVVHFFKNIVTPRTPPPS
MBP-3 (111-130)	LSRFSWGAEGQRPGFGYGGR
MBP-3.1 (111-129)	LSRFSWGAEGQRPGFGYGG
MBP-4 (141-165)	FKGVDAQGTLSKIFKLGGRDSRSGS
MBP-5 (101-125)	PSQGKGRGLSLSRFSWGAEGQRPGF

Fig. 3

1-20 A S Q K R P S Q R H G S K Y L A T A S T
11-30 G S K Y L A T A S T M D H A R H G F L P
21-40 M D H A R H G F L P R H R D T G I L D S
31-50 R H R D T G I L D S I G R F F G G D R G
41-60 I G R F F G G D R G A P K R G S G K D S
51-70 A P K R G S G K D S H H P A R T A H Y G
61-80 H H P A R T A H Y G S L P Q K S H G R T
71-90 S L P Q K S H G R T Q D E N P V V H F F
81-100 Q D E N P V V H F F K N I V T P R T P P
91-110 K N I V T P R T P P P S Q G K G R G L S
101-120 P S Q G K G R G L S L S R F S W G A E G
111-130 L S R F S W G A E G Q R P G F G Y G G R
121-140 Q R P G F G Y G G R A S D Y K S A H K G
131-150 A S D Y K S A H K G F K G V D A Q G T L
141-160 F K G V D A Q G T L S K I F K L G G R D
151-170 S K I F K L G G R D S R S G S P M A R R

82-100 D E N P V V H F F K N I V T P R T P P

83-105 E N P V V H F F K N I V T P R T P P P S Q G K

141-165
F K G V D A Q G T L S K I F K L G G R D S R S G S

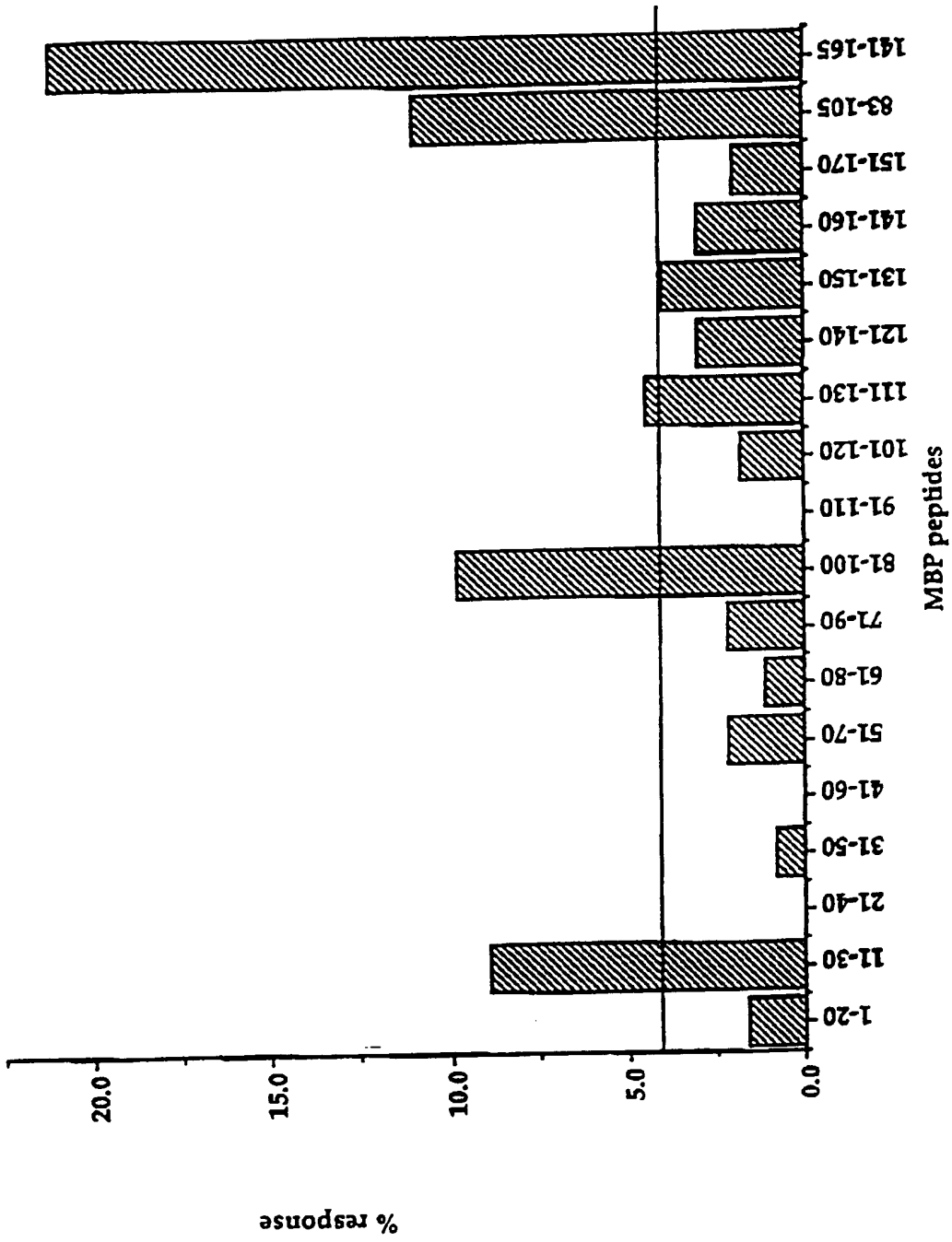

1-20
11-30
21-40
31-50
41-60
51-70
61-80
71-90
81-100
91-110
101-120
111-130
121-140
131-150
141-160
151-170
83-105
141-165
MBP peptides
0.0
5.0
10.0
15.0
20.0
% response

Fig. 4a


1-20
11-30
21-40
31-50
41-60
51-70
61-80
71-90
81-100
91-110
101-120
111-130
121-140
131-150
141-160
151-170
83-105
141-165
MBP peptides
0
10
20
30
40
50
60
% individuals

Fig. 4b

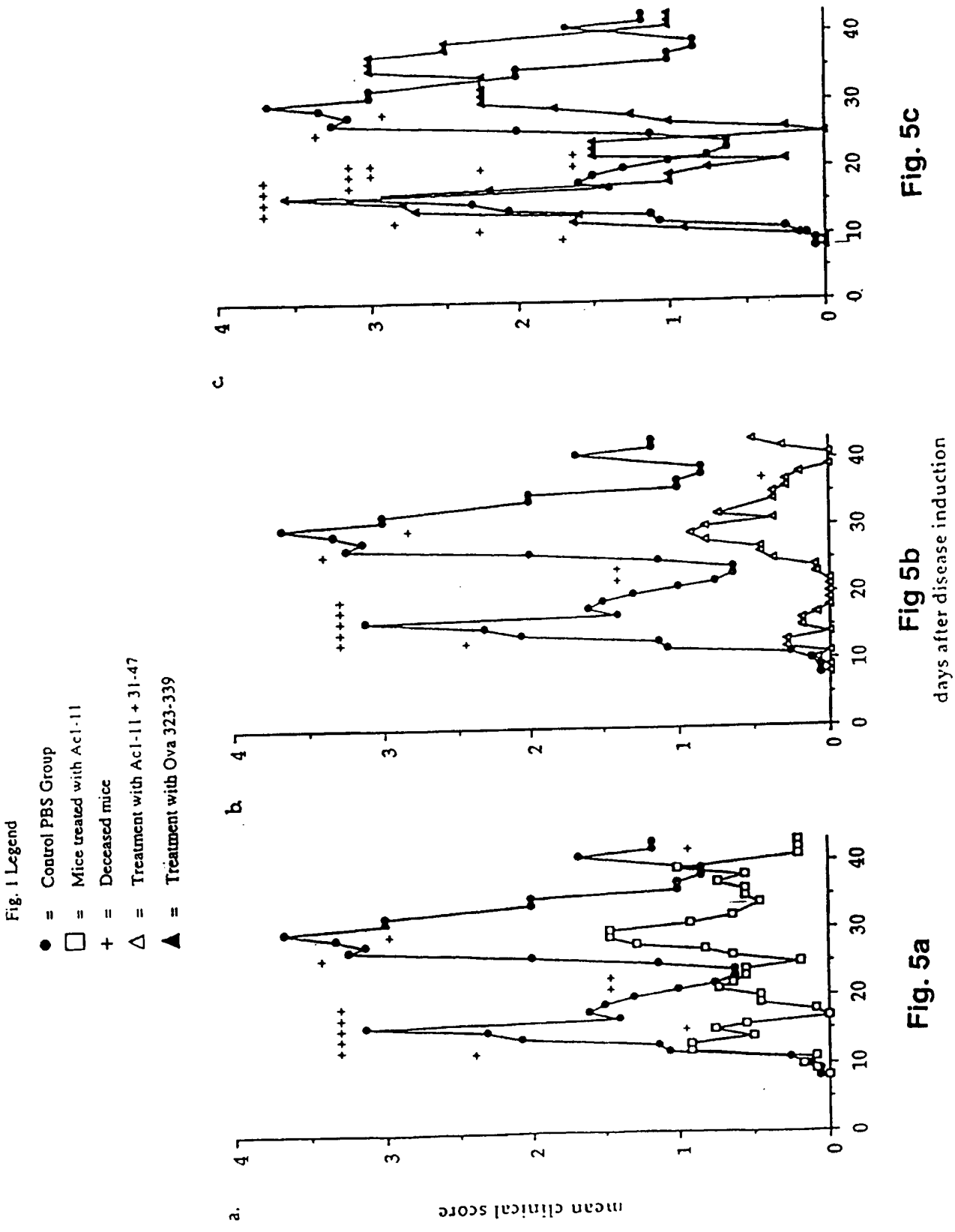


Fig. 5a

Fig 5b

Fig. 5c

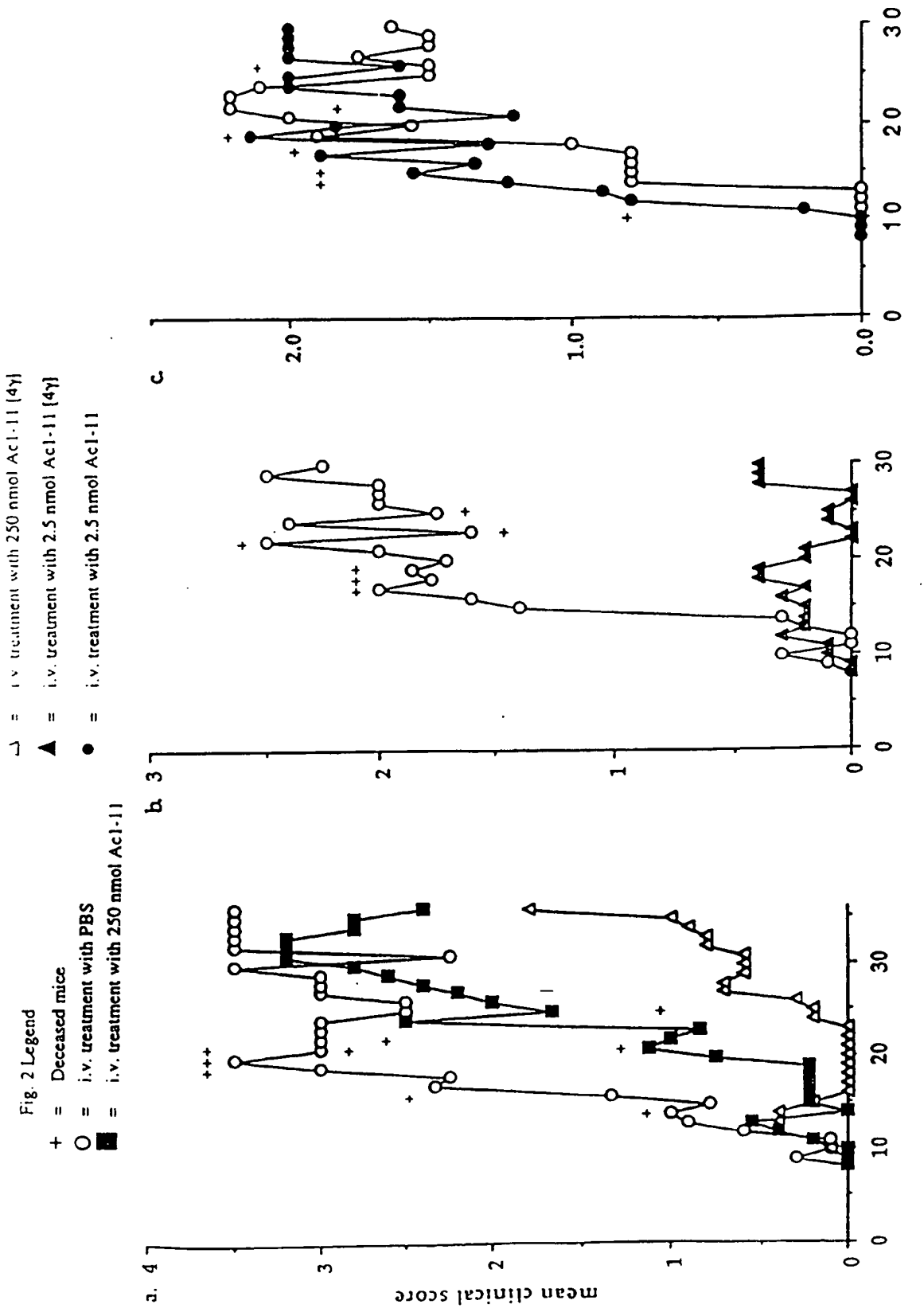
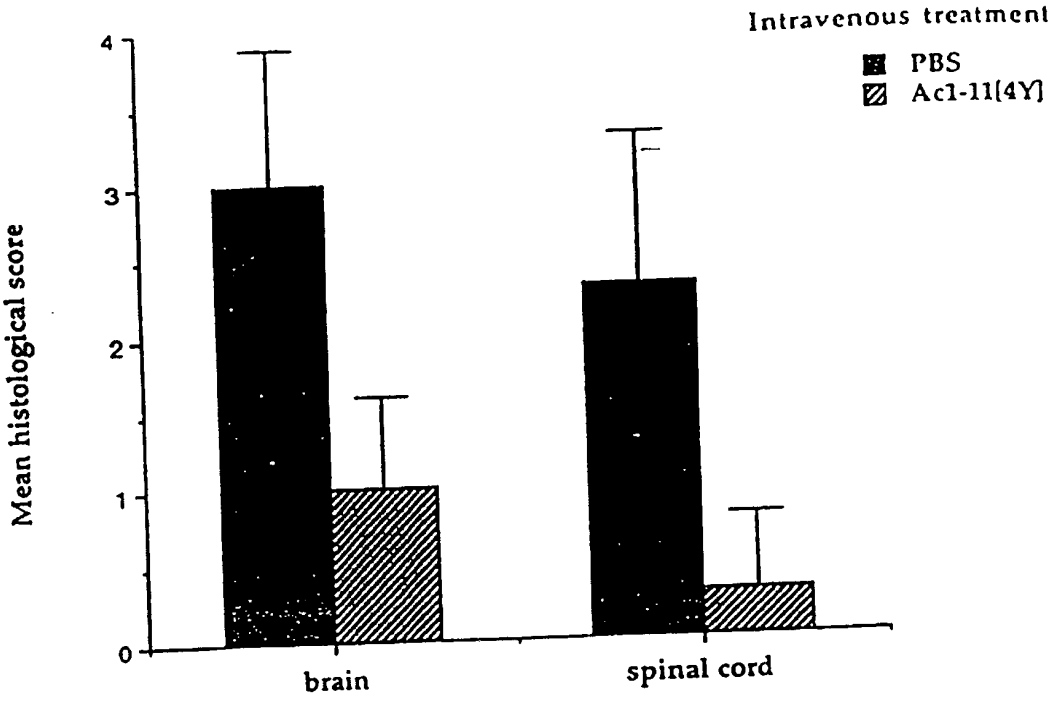


Fig. 6a

Fig. 6b

Fig. 6c

Fig. 7


Intravenous treatment
PBS
Ac1-11[4Y]
Mean histological score
0
1
2
3
4
brain
spinal cord

Human MBP

A S Q K R P S Q R H[10] G S K Y L A T A S T[20] M D H A R H G

F L P[30] R H R D T G I L D S[40] I G R F F G G D R G[50] A P K R G

S G K D S[60] H H P A R T A H Y G[70] S L P Q K S - H G R[80] T Q

D E N P V V H F[90] F K N I V T P R T P[100] P P S Q G K G R G

L[110] S L S R F S W G A E[120] G Q R P G F G Y G G[130] R A S D Y K

S A H K[140] G F K G V D A Q G T[150] L S K I F K L - G[160] G R D

S R S G S P M A[170] R R

Fig. 8

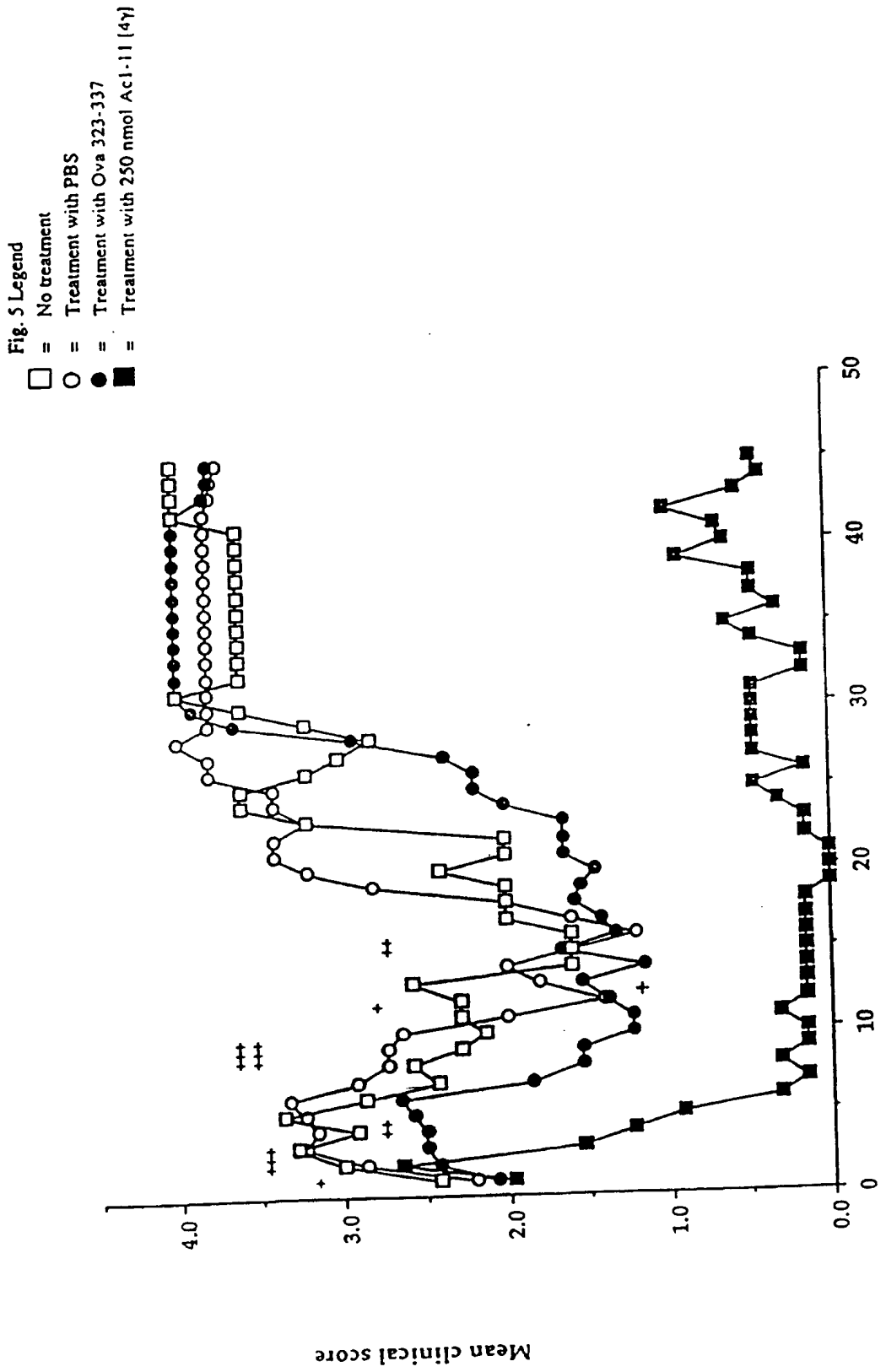


Fig. 5 Legend
□ = No treatment
○ = Treatment with PBS
● = Treatment with Ova 323-337
■ = Treatment with 250 nmol Ac1-11 [4Y]
Mean clinical score
4.0
3.0
2.0
1.0
0.0
0
10
20
30
40
50
Days following initiation of treatment
(day 1 = onset of clinical signs)

Fig. 9

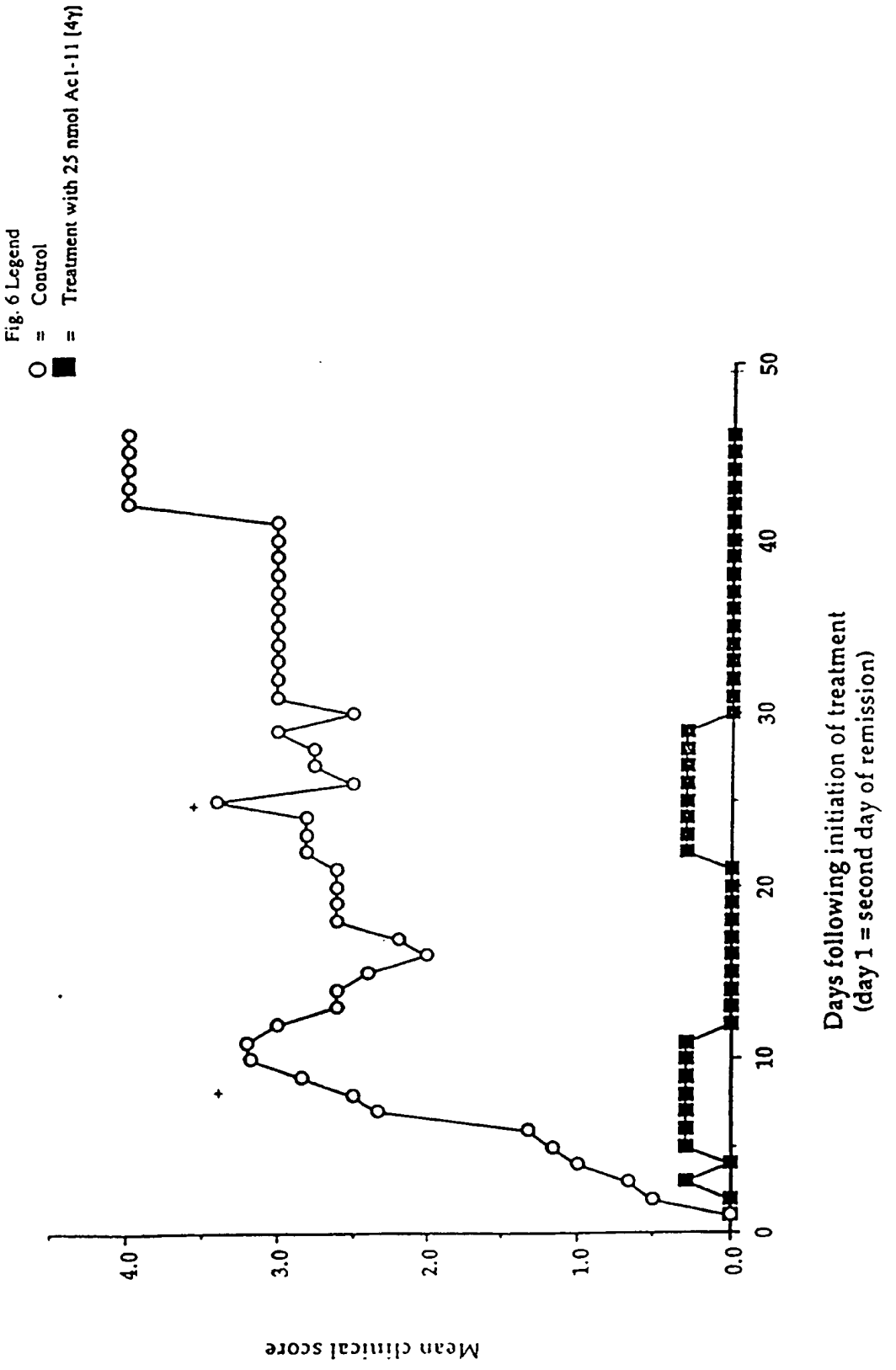


Fig. 6 Legend
○ = Control
■ = Treatment with 25 nmol Ac1-11 (4γ)
Mean clinical score
4.0
3.0
2.0
1.0
0.0
0
10
20
30
40
50
Days following initiation of treatment
(day 1 = second day of remission)

Fig. 10

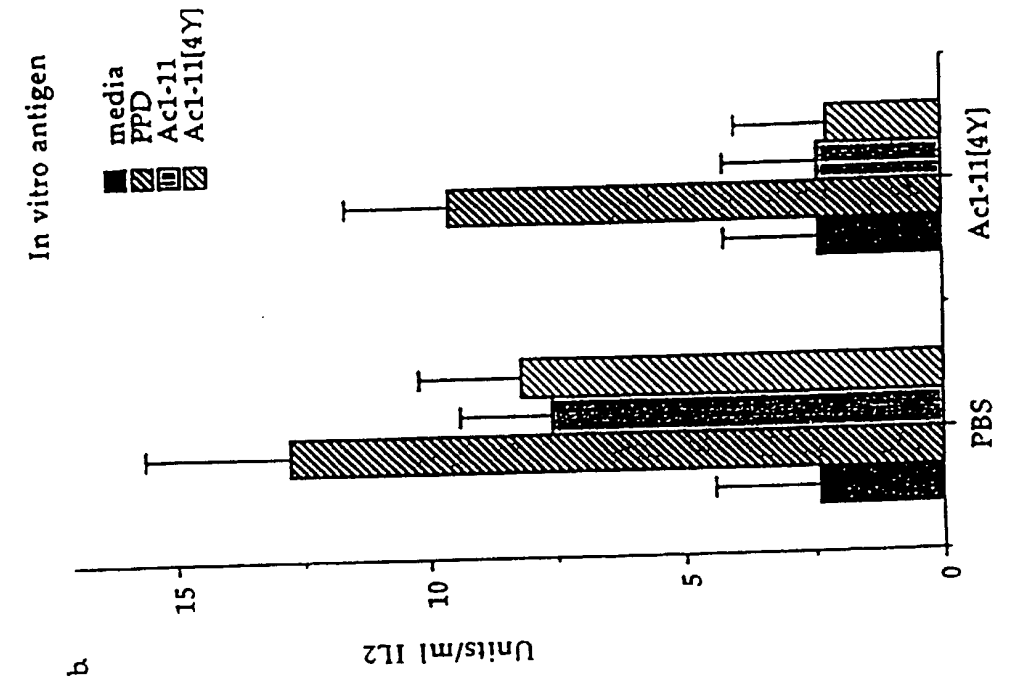


Fig. 11b

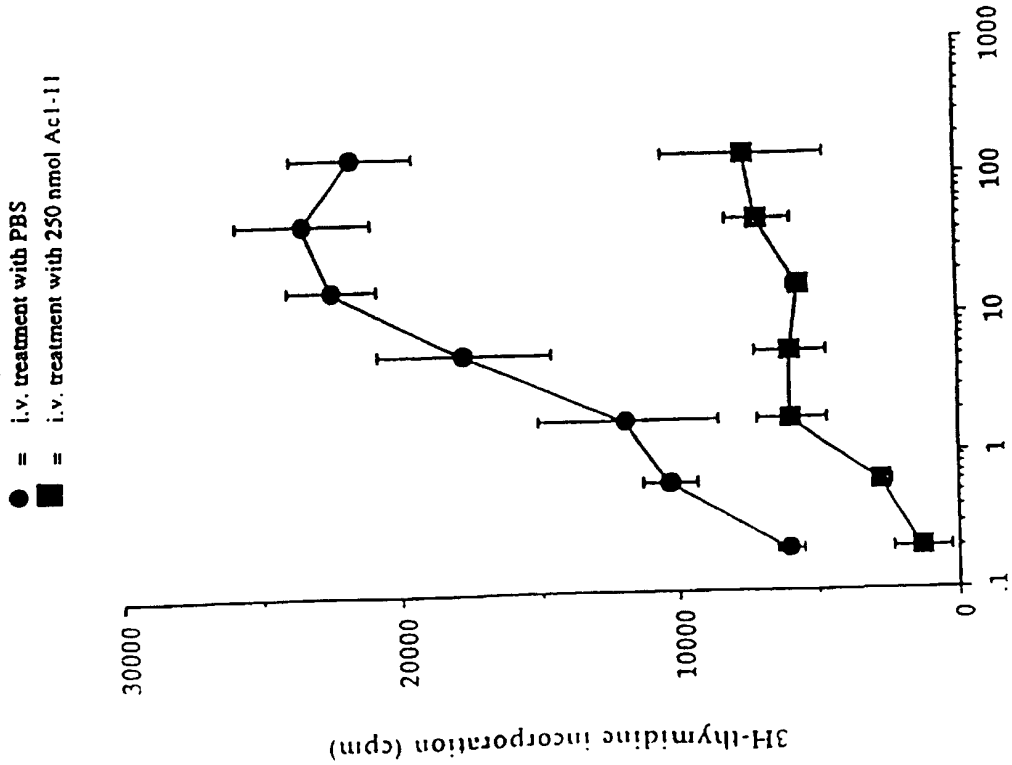


Fig. 11a

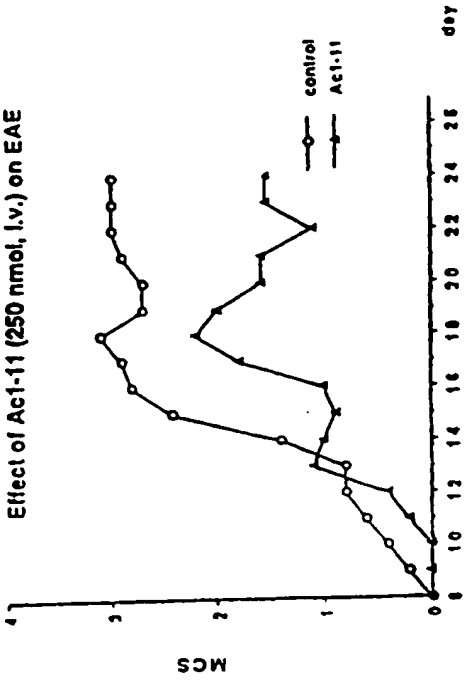


Fig. 12b

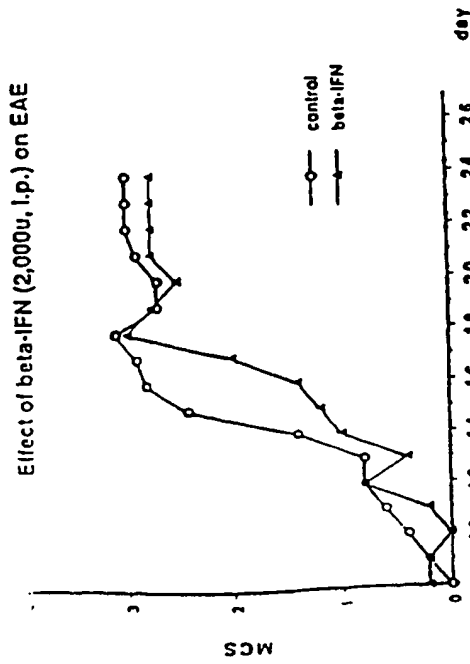


Fig. 12a

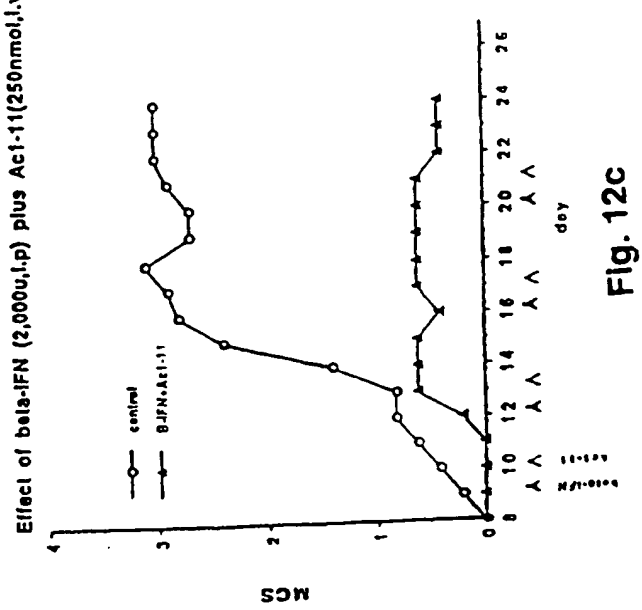


Fig. 12c

Fig. 13

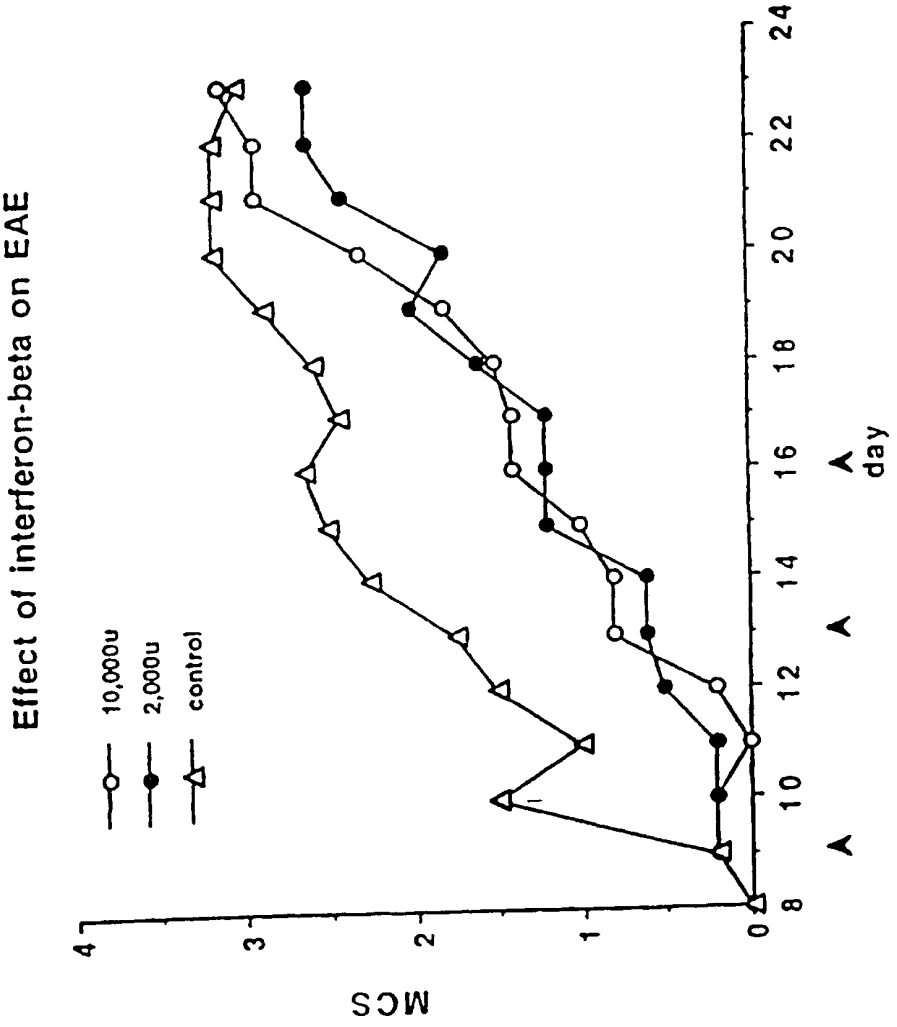

Effect of interferon-beta on EAE
10,000u
2,000u
control
MCS
day
0
1
2
3
4
8
10
12
14
16
18
20
22
24

Fig. 14

Amino Acid Residues	Amino Acid Sequences
13-25	KYLATASTMDHAR
31-50	RHRDTGILDSIGRFFGGDRG
61-80	HHPARTAHYGSLPQKSHGRT
82-92	DENPVVHFFKN
82-96	DENPVVHFFKNIVTP
82-97	DENPVVHFFKNIVTPR
82-98	DENPVVHFFKNIVTPRT
82-100	DENPVVHFFKNIVTPRTPP
82-100 [100 P>Y]	HFFΔNIVTPRTP
83-100	ENPVVHFFKNIVTPRTPP
83-101	ENPVVHFFKNIVTPRTPPP
84-97	NPVVHFFKNIVTPR
84-100	NPVVHFFKNIVTPRTPP
85-100	PVVHFFKNIVTPRTPP
86-105	VVHFFKNIVTPRTPPPSQGK
87-99	VHFFKNIVTPRTP
88-100	HFFKNIVTPRTPP
88-99	HFFKNIVTPRTP
82-100 [100 P>Y]	DENPVVHFFKNIVTPRTPY
111-135	LSRFSWGAEGQRPGFGYGGRASDYK
122-140	RPGFGYGGRASDYKSAHKG
139-170	KGFKGVDAQGTLSKIFKLGGRDSRSGSPMARR
141-160	FKGVDAQGTLSKIFKLGGRD
142-166	KGVDAQGTLSKIFKLGGRDSRSGSP
142-168	KGVDAQGTLSKIFKLGGRDSRSGSPMA
146-160	AQGTLSKIFKLGGRD
153-170	IFKLGGRDSRSGSPMARR

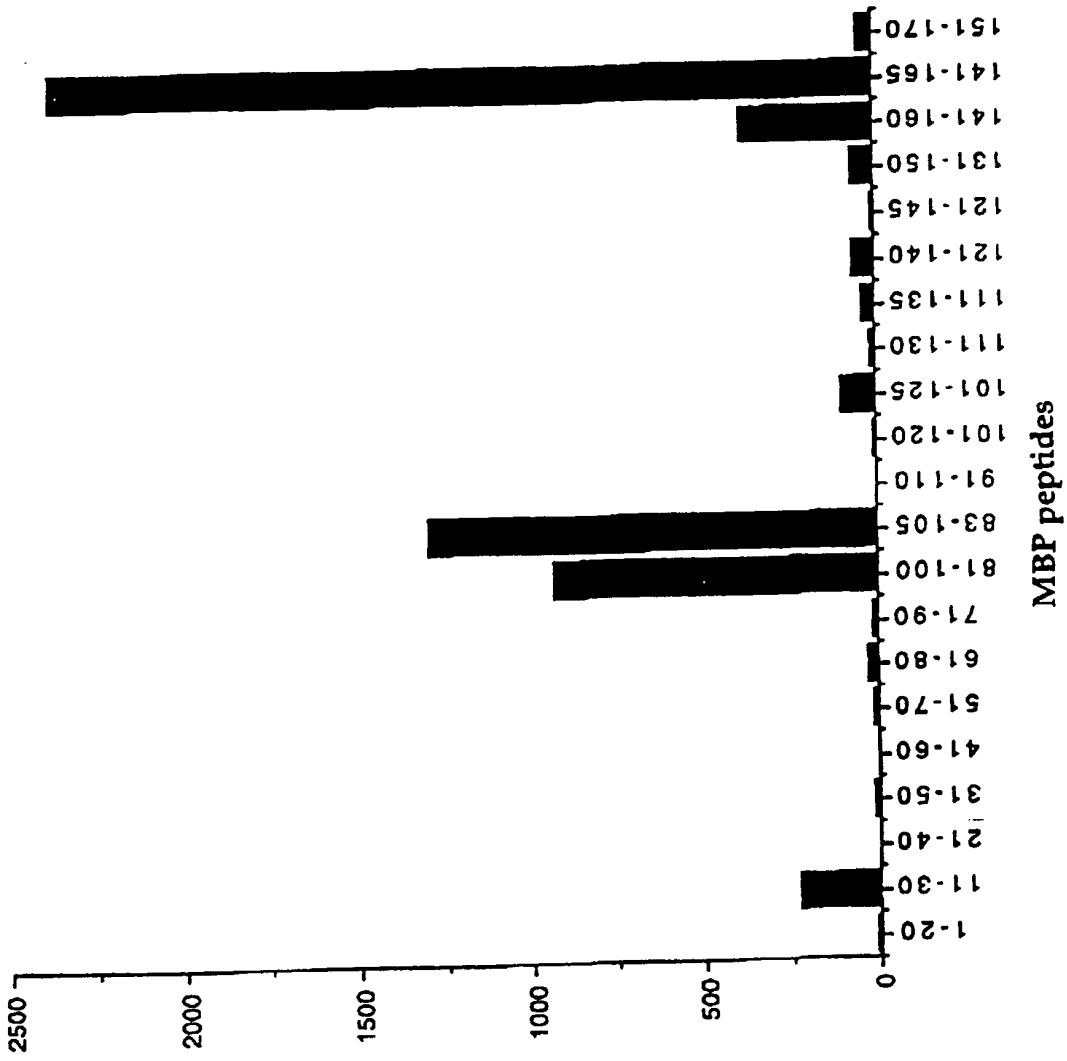

151-170
141-165
141-160
131-150
121-145
121-140
111-135
111-130
101-125
101-120
91-110
83-105
81-100
71-90
61-80
51-70
41-60
31-50
21-40
11-30
1-20
0
500
1000
1500
2000
2500
MBP peptides

Fig. 15

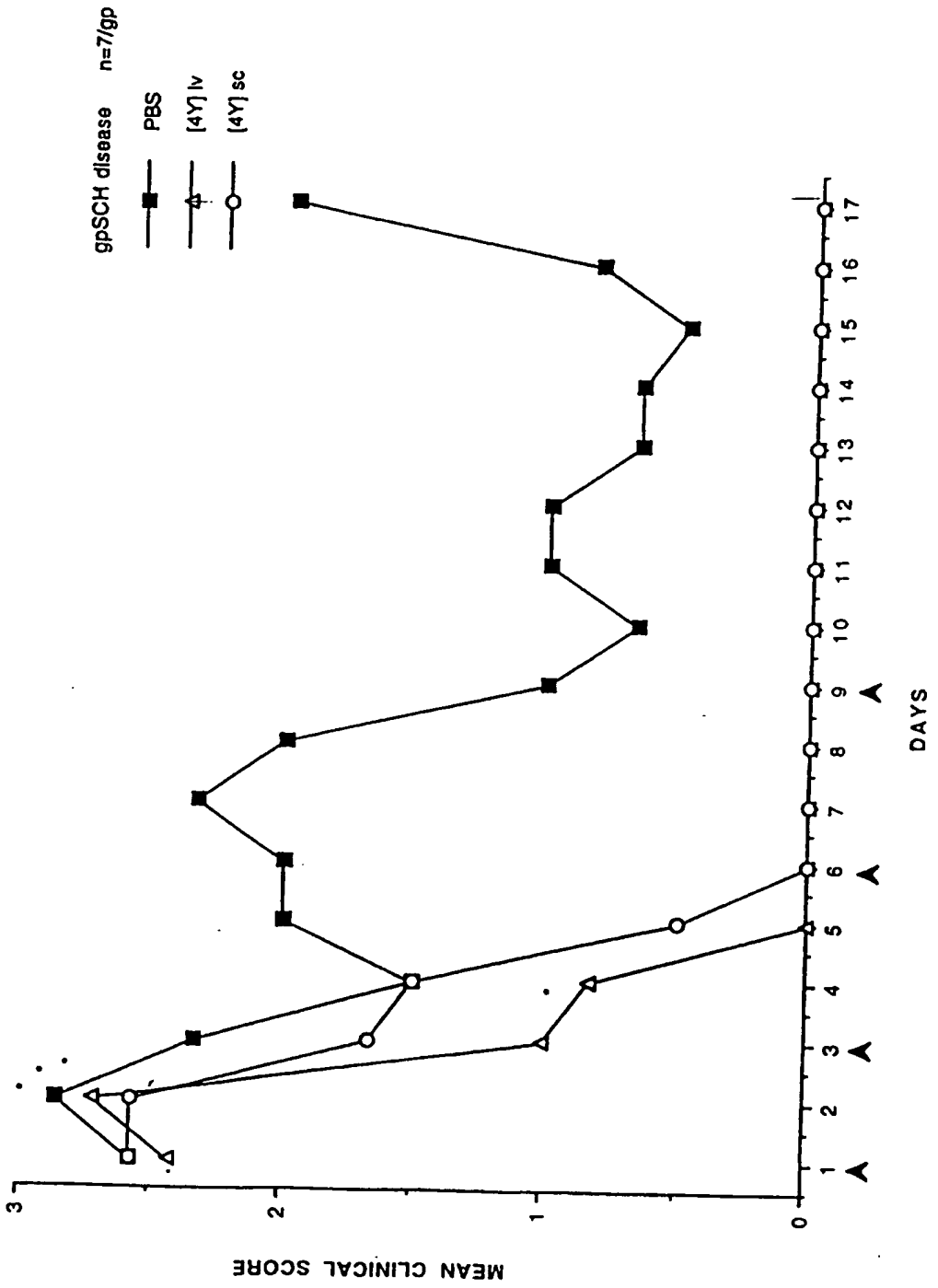

gpSCH disease n=7/gp
PBS
(4Y) iv
(4Y) sc
MEAN CLINICAL SCORE
0
1
2
3
DAYS
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

Fig. 16

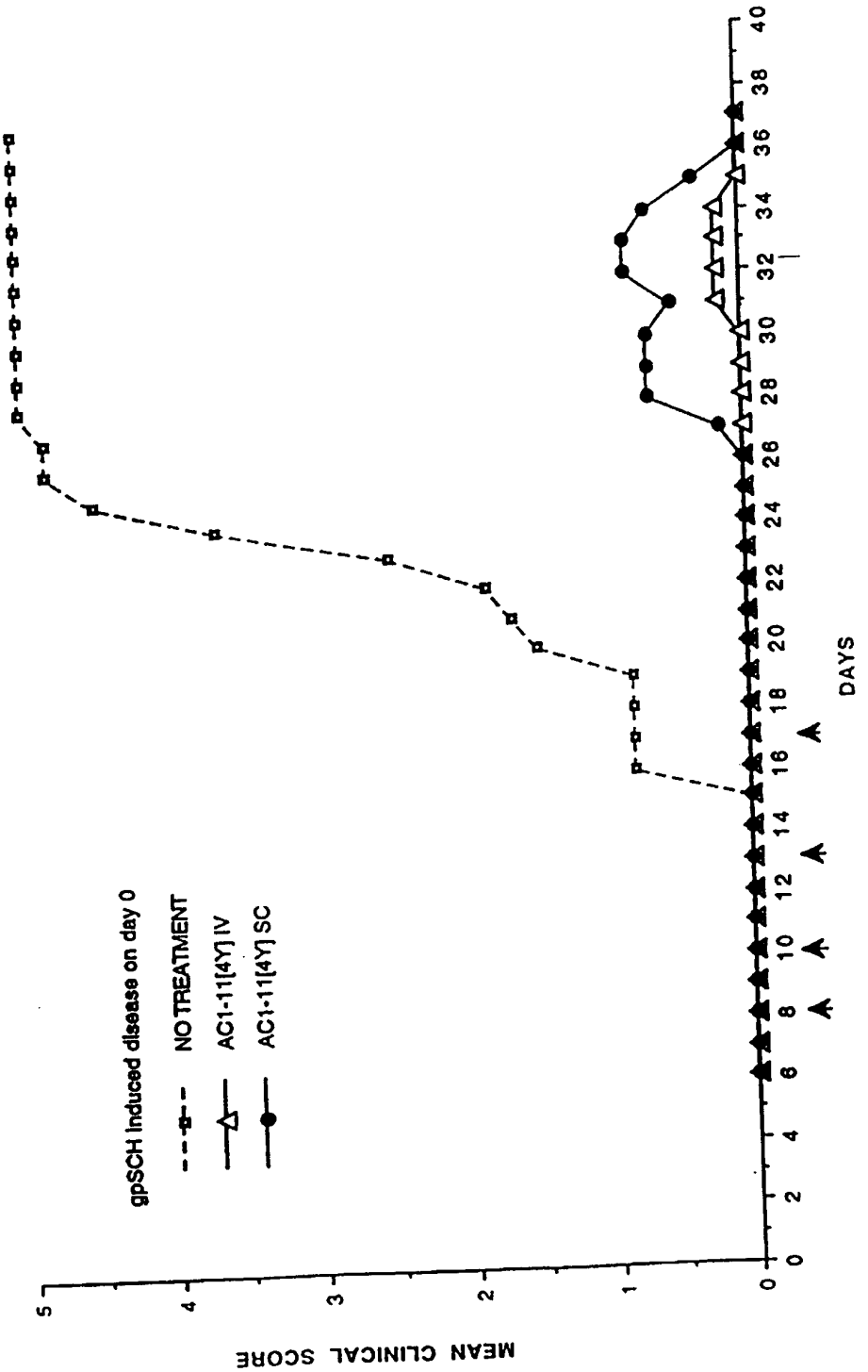

MEAN CLINICAL SCORE
0
1
2
3
4
5
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
DAYS
gpSCH induced disease on day 0
NO TREATMENT
AC1-11[4Y] IV
AC1-11[4Y] SC

Fig. 17

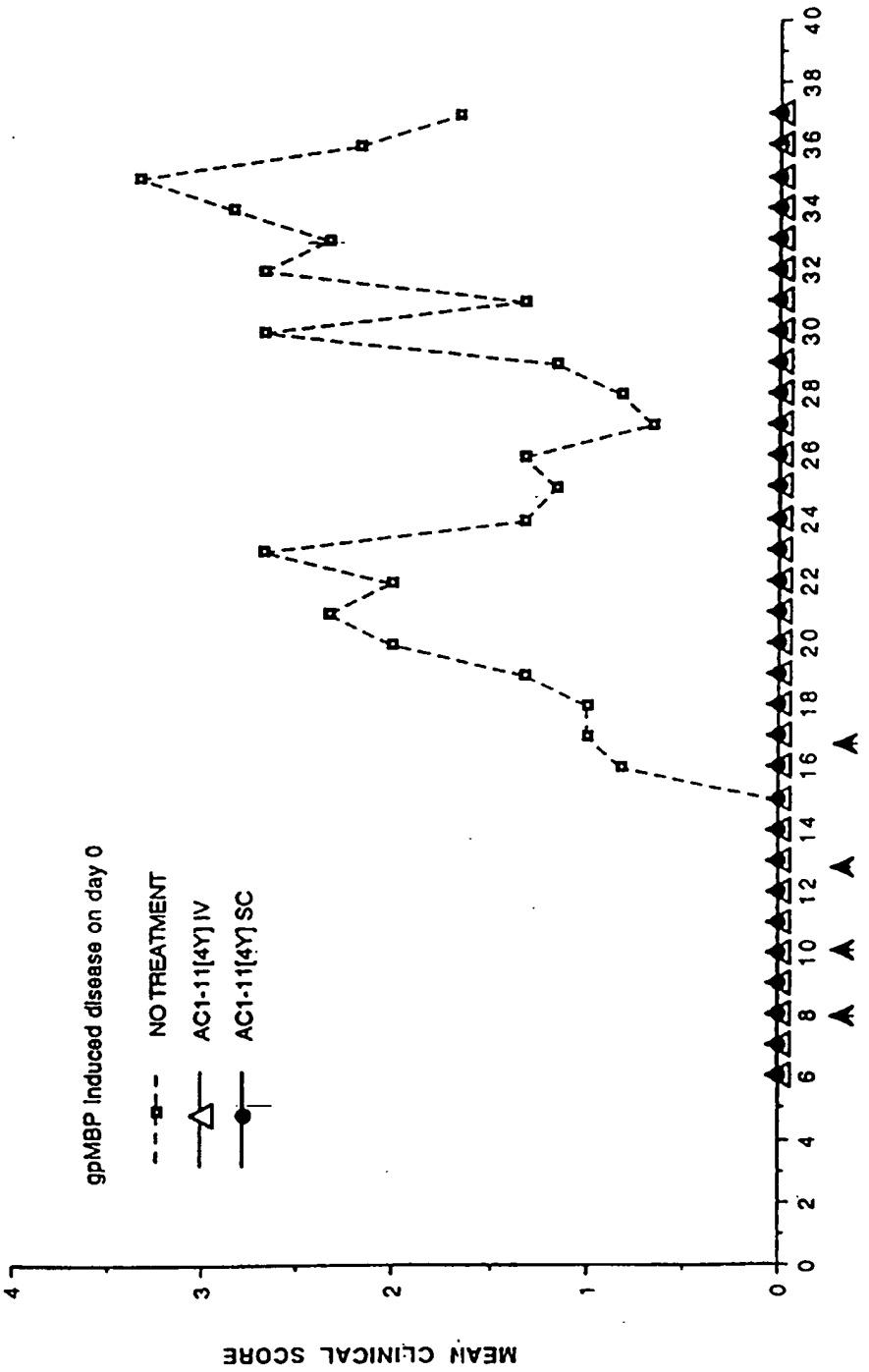

MEAN CLINICAL SCORE
0
1
2
3
4
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40
DAYS
gpMBP Induced disease on day 0
NO TREATMENT
AC1-11[4Y] IV
AC1-11[4Y] SC

Fig. 18

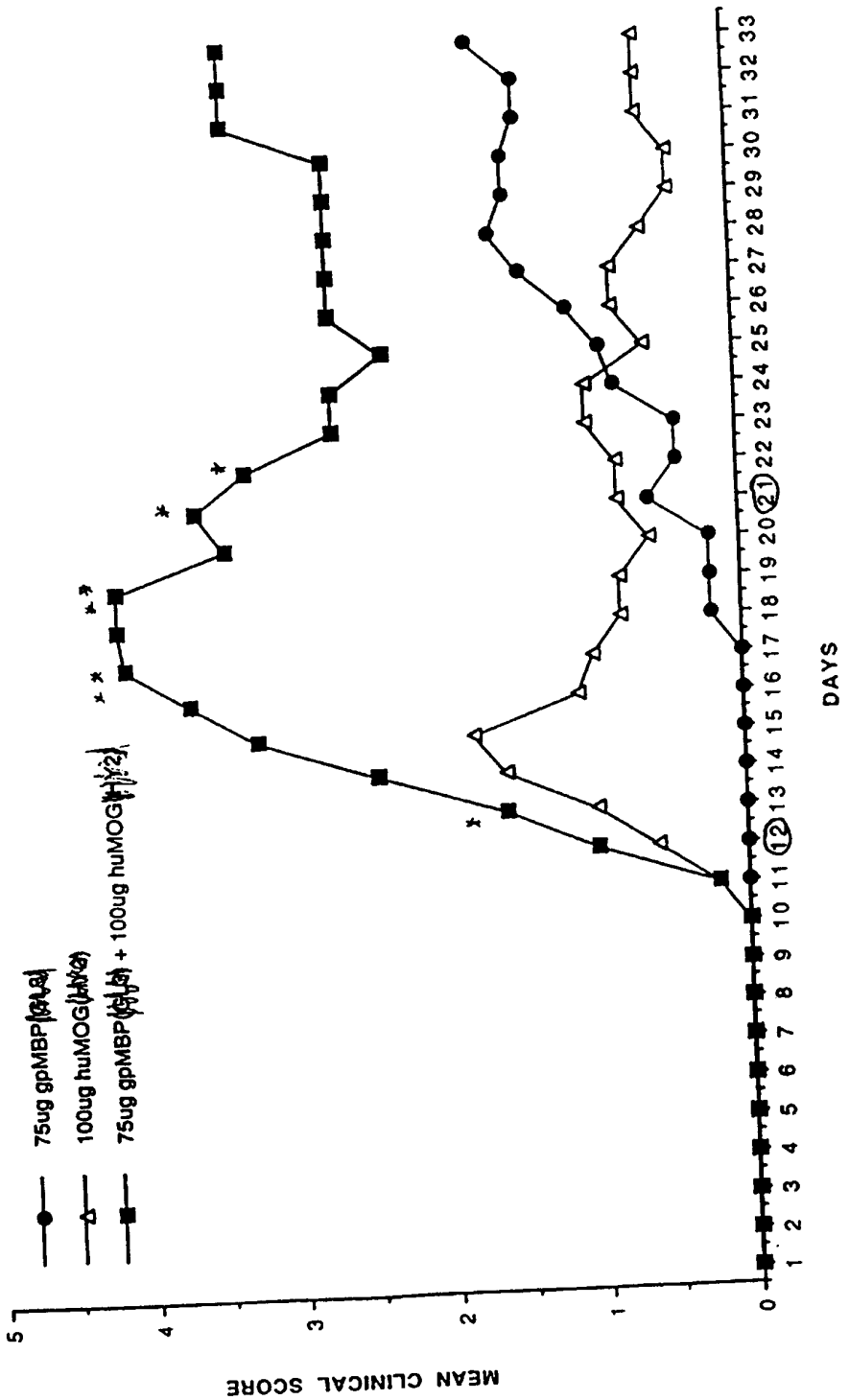

MEAN CLINICAL SCORE
DAYS
75ug gpMBP
100ug huMOG
75ug gpMBP + 100ug huMOG

Fig. 19

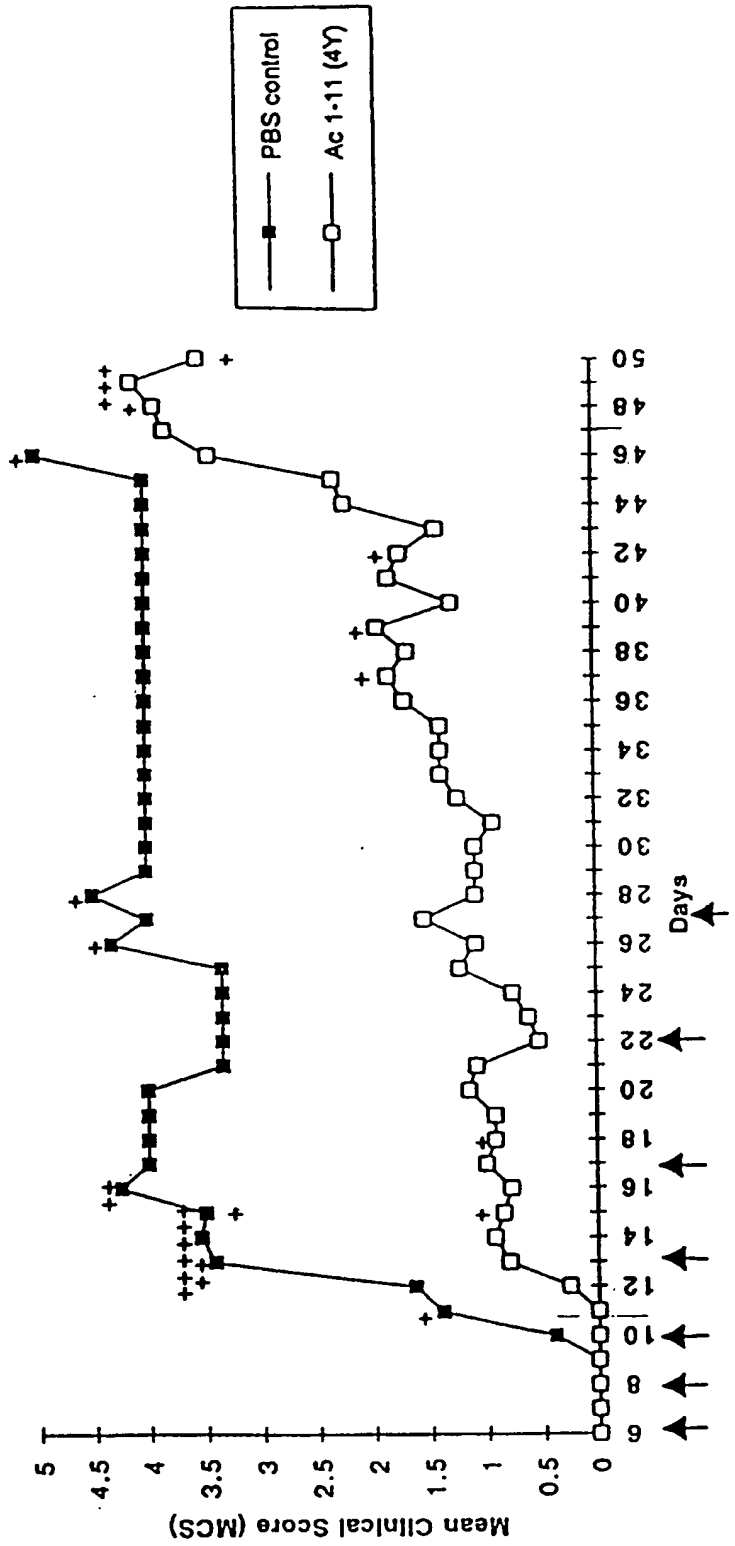

PBS control
Ac 1-11 (4Y)
Mean Clinical Score (MCS)
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Days
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50

Fig. 20

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